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Set	Items	Description
S1	2015	THURINGIENSIS
S2	6	CRYIG OR CRYI(10W)G

2/6/1 09744970 98435172
A new PCR-based approach to a fast search of a wide spectrum of cry genes from Bacillus thuringiensis strains. Sep 1998

2/6/2 09701646 98381841
Limited proteolysis of Bacillus thuringiensis CryIG and CryIVB delta-endotoxins leads to formation of active fragments that do not coincide with the structural domains. Jul 1998

2/6/3 08141691 95219756
Production of multiple delta-endotoxins by Bacillus thuringiensis: delta-endotoxins produced by strains of the subspecies galleriae and wuhanensis. Dec 1994

2/6/4 07969734 94329085
[Multiple genes of delta-endotoxins from Bacillus thuringiensis subspecies galleriae] Mnozhestvennye geny delta-endotoksinov Bacillus thuringiensis podvida galleriae. May-Jun 1994

2/6/5 07704237 94085596
Primary structure of cryX*, the novel delta-endotoxin-related gene from Bacillus thuringiensis spp. galleriae. Dec 20 1993

2/6/6 06953438 92070568
Nucleotide sequence of a novel delta-endotoxin gene cryIg of Bacillus thuringiensis ssp. galleriae. Nov 18 1991

2/5/5 DIALOG(R)File 155:MEDLINE(R) (c) format only 1999 Dialog Corporation. All rts. reserv.
07704237 94085596
Primary structure of cryX*, the novel delta-endotoxin-related gene from Bacillus thuringiensis spp. galleriae.
Shevelev AB; Svarinsky MA; Karasin AI; Kogan YaN; Chestukhina GG; Stepanov VM
Institute of Microbial Genetics (VNIIGenetika), Laboratory of Protein Chemistry, Moscow, Russian Federation.
FEBS Lett (NETHERLANDS) Dec 20 1993, 336 (1) p79-82, ISSN 0014-5793 Journal Code: EUH Languages: ENGLISH Document type: JOURNAL ARTICLE JOURNAL ANNOUNCEMENT: 9403 Subfile: INDEX MEDICUS
A cry-related sequence, designated cryX (EMBL X75019), was localized upstream of cryIG, the delta-endotoxin gene cloned from spp. galleriae of Bacillus thuringiensis and sequenced earlier [(1991) FEBS Lett. 293, 25-28]. Analysis of the cryX complete nucleotide sequence enabled us to explain its virtual crypticity and to reveal the chimeric structure of the genes, cryX and cryIG. The amino acid sequence of 1,151 residues encoded by the continuous reading frame of cryX is similar to the other delta-endotoxins but differs essentially from them
. Tags: Support, Non-U.S. Gov't Descriptors: *Bacillus thuringiensis--Genetics--GE; *Bacterial Proteins --Genetics--GE; *Endotoxins--Genetics--GE; *Genes, Bacterial; Amino Acid Sequence; Bacillus thuringiensis--Metabolism--ME; Base Sequence; Chimera; DNA, Recombinant; Molecular Sequence Data
CAS Registry No.: 0 (cryX protein); 0 (Bacillus thuringiensis crystal protein); 0 (Bacterial Proteins); 0 (DNA, Recombinant); 0 (Endotoxins) Gene Symbol: cryX ?

2/7/6 DIALOG(R)File 155:MEDLINE(R) (c) format only 1999 Dialog Corporation. All rts. reserv.
06953438 92070568
Nucleotide sequence of a novel delta-endotoxin gene cryIg of Bacillus thuringiensis ssp. galleriae.
Smulevitch SV; Osterman AL; Shevelev AB; Kaluger SV; Karasin AI; Kadyrov RM; Zagnitko OP; Chestukhina GG; Stepanov VM
Institute of Microbial Genetics, Lab. of Protein Chemistry, Moscow, USSR.
FEBS Lett (NETHERLANDS) Nov 18 1991, 293 (1-2) p25-8, ISSN 0014-5793 Journal Code: EUH Languages: ENGLISH Document type: JOURNAL ARTICLE
A gene cryIg coding for entomocidal protein delta-endotoxin of Bacillus thuringiensis ssp. galleriae str. 11-67 named CryIG has been cloned and sequenced (EMBL accession number X58120). The deduced amino acid sequence that contains 1156 amino acid residues shows only 28% of identical residues, when compared with other delta-endotoxins of the CryI family. The extent of identity is substantially higher for some regions of the sequence ('conserved blocks'), that presumably bear important structural or functional properties. This implies that CryIG delta-endotoxin follows the same type of polypeptide chain folding as other CryI proteins, whereas peculiarities of primary structure help to explain its unique specificity.
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(FILE 'USPAT' ENTERED AT 08:28:28 ON 17 JUN 1999)
L1 1220 S THURINGIENSIS
L2 7890 S TOXIN OR ENDOTOXIN
L3 510 S L1(P)L2
L4 160995 S FUS? OR CHIMER?
L5 767 S L2(P)L4
L6 128 S L5 AND L3
L7 38251 S L4/TI,AB,CLM
L8 55 S L7 AND L6
L9 378 S L1/TI,AB,CLM
L10 31 S L8 AND L9
L11 4 S CRYIG/CRYIC OR CRYIG(3N)CRYIC
L12 17 S CRYIE(3N)CRYIC
L13 2 S L4(P)L12
L14 21 S CRYIA(3N)CRYIC
L15 8 S L4(P)L14
L16 39618 S HYBRID? NOT HYBRIDI?
WARNING - PROXIMITY OPERATOR PRECEDENCE LEVEL CONFLICTS OR IS NOT CONSISTENT WITH FIELD CODE - 'AND' OPERATOR ASSUMED 'L16(P)L12'
L17 5 S L16(P)L12
WARNING - PROXIMITY OPERATOR PRECEDENCE LEVEL CONFLICTS OR IS NOT CONSIS TENT WITH FIELD CODE - 'AND' OPERATOR ASSUMED 'L16(P)L14'
L18 4 S L16(P)L14

l6 1. 5,908,970, Jun. 1, 1999, Recombinant plant expressing non-competitively binding Bt insecticidal crystal proteins; Herman Van Mellaert, et al., 435/320.1, 419; 536/23.71 [IMAGE AVAILABLE]

2. 5,889,174, Mar. 30, 1999, Nucleotide sequences encoding pesticidal proteins; Gregory W. Warren, et al., 536/23.71; 435/69.1; 536/23.7 [IMAGE AVAILABLE]

3. 5,888,801, Mar. 30, 1999, Pesticidal strains of bacillus; Gregory W. Warren, et al., 435/252.5; 424/93.46 [IMAGE AVAILABLE]

4. 5,885,603, Mar. 23, 1999, Insecticidal matrix and process for preparation thereof; Jeffrey D. Fowler, et al., 424/405, 408, 409, 417, 421, 485, 491, 496, 497, 499, 500, 501 [IMAGE AVAILABLE]

5. 5,880,328, Mar. 9, 1999, DNA encoding plant chitinases; John A. Ryals, et al., 800/298; 435/69.1, 200, 209, 320.1, 418, 419; 536/23.2, 23.6; 800/301, 302, 317.3 [IMAGE AVAILABLE]

6. 5,880,275, Mar. 9, 1999, Synthetic plant genes from BT kurstaki and method for preparation; David A. Fischhoff, et al., 536/23.71, 23.6 [IMAGE AVAILABLE]

7. 5,877,012, Mar. 2, 1999, Class of proteins for the control of plant pests; Juan J. Estruch, et al., 435/252.3, 235.1, 252.31, 252.32, 252.33, 252.34, 252.35, 254.11, 257.2, 320.1; 530/350; 536/23.71 [IMAGE AVAILABLE]

8. 5,874,662, Feb. 23, 1999, Method for producing somoclonal variant cotton plants; Thirumale S. Rangan, et al., 800/276; 435/418, 427, 430.1, 431; 800/265, 268, 270, 298, 301, 314 [IMAGE AVAILABLE]

9. 5,874,298, Feb. 23, 1999, Insecticidal toxins from Bracon hebetor nucleic ac[illegible] encoding said toxin and methods of use; Janice H. Johnson, et al., 435/325, 69.1, 320.1; 514/12; 530/350; 536/23.1 [IMAGE AVAILABLE]

10. 5,874,288, Feb. 23, 1999, Bacillus thuringiensis toxins with improved activity; Mark Thompson, et al., 435/252.3; 424/93.2; 435/252.33, 252.34, 320.1, 419; 536/23.1, 23.71; 800/302 [IMAGE AVAILABLE]

11. 5,872,212, Feb. 16, 1999, Pesticidal proteins and strains; Gregory W. Warren, et al., 530/350, 825 [IMAGE AVAILABLE]

12. 5,866,784, Feb. 2, 1999, Recombinant plant expressing non-competitively binding insecticidal crystal proteins; Herman Van Mellaert, et al., 800/302; 435/320.1, 419, 430; 536/23.71 [IMAGE AVAILABLE]

13. 5,866,326, Feb. 2, 1999, Method for isolating vegetative insecticidal protein genes; Gregory W. Warren, et al., 435/6, 91.1; 536/25.4 [IMAGE AVAILABLE]

14. 5,859,347, Jan. 12, 1999, Enhanced expression in plants; Sherri Marie Brown, et al., 800/278; 435/69.1, 70.1, 320.1; 536/23.1, 24.1; 800/279, 280, 300, 300.1, 301, 302, 320.1, 320.2, 320.3 [IMAGE AVAILABLE]

15. 5,859,336, Jan. 12, 1999, Synthetic DNA sequence having enhanced activ[illegible] maize; Michael G. Koziel, et al., 800/302; 435/69.1, 320.1, 418, 419; 536/23.71, 24.1; 800/287, 320.1 [IMAGE AVAILABLE]

16. 5,859,328, Jan. 12, 1999, Isolated DNA elements that direct pistil-specific and anther-specific gene expression and methods of using same; Mikhail E. Nasrallah, et al., 800/287; 435/69.1, 70.1, 320.1; 536/24.1; 800/286, 294, 298, 303 [IMAGE AVAILABLE]

17. 5,859,321, Jan. 12, 1999, Cotton somaclonal variants; Thirumale S. Rangan, et al., 800/301; 435/418, 420, 427, 430, 430.1, 431; 800/314 [IMAGE AVAILABLE]

18. 5,858,745, Jan. 12, 1999, Bacillus thuringiensis transformation method; Cindy Lou Jellis, et al., 435/485, 173.1, 173.3, 173.6, 471 [IMAGE AVAILABLE]

19. 5,856,177, Jan. 5, 1999, Promoters derived from the maize phosphoenolpyruvate carboxylase gene involved in C.sub.4 photosynthesis; John W.

Grula, et al., 435/320.1, 69.1, 419, 468, 469; 536/23.2, 23.6, 24.1 [IMAGE AVAILABLE]

20. 5,856,154, Jan. 5, 1999, Method of protecting plants from oomycete pathogens; John A. Ryals, et al., 800/279; 435/69.1, 418, 419; 536/23.6 [IMAGE AVAILABLE]

21. 5,851,766, Dec. 22, 1998, Process for isolating chemically regulatable DNA sequences; John A. Ryals, et al., 435/6, 91.2 [IMAGE AVAILABLE]

22. 5,851,545, Dec. 22, 1998, Insecticidal matrix and process for preparation thereof; Jeffrey D. Fowler, et al., 424/405, 93.3, 409, 461, 486, 487 [IMAGE AVAILABLE]

23. 5,849,870, Dec. 15, 1998, Pesticidal proteins and strains; Gregory W. Warren, et al., 530/350; 435/252.31, 252.5, 320.1; 536/23.1, 23.7, 23.71 [IMAGE AVAILABLE]

24. 5,847,258, Dec. 8, 1998, DNA encoding .beta.-1,3-glucanases; John A. Ryals, et al., 800/301; 435/69.1, 209, 320.1, 418, 419; 536/23.6, 24.1; 800/298 [IMAGE AVAILABLE]

25. 5,843,898, Dec. 1, 1998, Transformation vectors allowing expression of foreign polypeptide endotoxins in plants; Henri Marcel Jozef De Greve, et al., 514/12; 435/69.1 [IMAGE AVAILABLE]

26. 5,843,744, Dec. 1, 1998, Bacillus thuringiensis Tn5401 proteins; James A. Baum, 435/183, 196 [IMAGE AVAILABLE]

27. 5,843,711, Dec. 1, 1998, Diphtheria toxin receptor-binding region; R. John Collier, et al., 435/69.1, 69.3, 252.3, 320.1; 514/2; 536/22.1, 23.1, 23.2, 23.4, 23.7 [IMAGE AVAILABLE]

28. 5,840,868, Nov. 24, 1998, Pesticidal proteins and strains; Gregory W. Warren, et al., 536/23.1; 435/6, 320.1; 530/350; 536/24.1 [IMAGE AVAILABLE]

29. 5,840,554, Nov. 24, 1998, .beta.-Endotoxin expression in pseudomonas fluorescens; Mark Thompson, et al., 435/471; 424/405, 538; 435/69.7, 252.34, 320.1, 480; 514/2; 530/350; 536/23.4, 23.71 [IMAGE AVAILABLE]

30. 5,834,292, Nov. 10, 1998, Method for producing somaclonal variant cotton plants; Thirumale S. Rangan, et al., 800/268; 435/427 [IMAGE AVAILABLE]

31. 5,827,514, Oct. 27, 1998, Pesticidal compositions; Gregory A. Bradfisch, et al., 424/93.2, 93.1, 93.3; 435/69.1, 69.7, 252.3, 410, 418, 419 [IMAGE AVAILABLE APPL-NO: 08/598,305 DATE FILED: Feb. 8, 1996 REL-US-DATA: Continuation of Ser. No. 349,867, Dec. 6, 1994, Pat. No. 5,508,264.

32. 5,824,542, Oct. 20, 1998, Methods for the production of hybrid seed; Lyle D. Crossland, et al., 435/320.1; 47/DIG.1; 435/69.1, 419; 536/23.6, 23.7, 24.1, 24.5 [IMAGE AVAILABLE]

33. 5,824,302, Oct. 20, 1998, Method of controlling insect larvae comprising feeding an insecticidal amount of a transgenic maize plant expressing a polypeptide having Bt-crystal protein toxic properties; Gleta Carswell, et al., 424/93.21, 93.2, 93.461; 536/23.71; 800/302 [IMAGE AVAILABLE]

34. 5,804,693, Sep. 8, 1998, Chemically regulatable and anti-pathogenic DNA sequences and uses thereof; Thomas D. Gaffney, et al., 800/301; 424/9.2; 435/29, 419; 800/298, 300, 302 [IMAGE AVAILABLE]

35. 5,804,393, Sep. 8, 1998, Antibodies directed to the binding proteins of Bacillus thuringiensis and their use; Martin Geiser, et al., 435/7.2, 7.32, 7.92, 7.93, 975; 436/501, 503, 547, 548, 808; 530/387.1, 387.2, 388.22, 389.1 [IMAGE AVAILABLE]

36. 5,789,214, Aug. 4, 1998, Method of inducing gene transcription in a plant; John A. Ryals, et al., 800/288; 435/418, 419; 536/23.6, 24.1 [IMAGE AVAILABLE]

37. 5,780,708, Jul. 14, 1998, Fertile transgenic corn plants; Ronald C. Lundquist, et al., 800/300.1, 301, 302, 320.1 [IMAGE AVAILABLE]

38. 5,777,200, Jul. 7, 1998, Chemically regulatable and anti-pathogenic DNA sequences and uses thereof; John A. Ryals, et al., 435/6, 91.51 [IMAGE AVAILABLE]

39. 5,776,449, Jul. 7, 1998, Recombinant bacillus thuringiensis strains, insecticidal compositions and method of use; James A. Baum, 424/93.2, 93.461, 405; 435/170, 252.31, 832 [IMAGE AVAILABLE]

40. 5,773,705, Jun. 30, 1998, Ubiquitin fusion protein system for protein production in plants; Richard David Vierstra, et al., 800/294; 435/69.1, 69.7, 320.1, 419, 468, 469, 470; 536/23.4, 23.6; 800/279, 298, 302 [IMAGE AVAILABLE]

41. 5,770,696, Jun. 23, 1998, Auxiliary proteins for enhancing the insecticidal activity of pesticidal proteins; Gregory W. Warren, et al., 530/350, 825; 536/23.1, 23.7, 23.71 [IMAGE AVAILABLE]

42. 5,770,450, Jun. 23, 1998, Zea mays plants regenerated from protoplasts or protoplast-derived cells; Ray Shillito, et al., 435/424, 412, 421, 430.1, 431 [IMAGE AVAILABLE]

43. 5,770,192, Jun. 23, 1998, Biological control agents; Patricia Jane Cayley, et al., 424/93.2, 93.6; 435/235.1, 320.1 [IMAGE AVAILABLE]

44. 5,767,372, Jun. 16, 1998, Transformation vectors allowing expression of foreign polypeptide endotoxins from Bacillus thuringiensis in plants; Henri Marcel Jozef De Greve, et al., 800/302; 435/320.1, 419; 536/23.71 [IMAGE AVAILABLE]

45. 5,767,369, Jun. 16, 1998, DNA sequences encoding SAR8.2 proteins and uses thereof; John A. Ryals, et al., 800/279; 435/69.1, 320.1, 418, 419; 536/23.6; 800/301 [IMAGE AVAILABLE]

46. 5,766,900, Jun. 16, 1998, Method of regenerating fertile transgenic Zea mays plants from protoplasts; Ray Shillito, et al., 800/292; 47/DIG.1 [IMAGE AVAILABLE]

47. 5,766,625, Jun. 16, 1998, Artificial viral envelopes; Hans Schreier, et al., 424/450; 264/4.1, 4.3; 424/192.1, 204.1, 208.1, 812; 436/829 [IMAGE AVAILABLE]

48. 5,763,241, Jun. 9, 1998, Insect resistant plants; David A. Fischhoff, et al., 800/279; 435/418, 419; 536/23.71 [IMAGE AVAILABLE]

49. 5,760,181, Jun. 2, 1998, Endotoxins; Henri Marcel Jozef De Greve, et al., 530/350 [IMAGE AVAILABLE]

50. 5,756,459, May 26, 1998, Insecticidally effective peptides isolatable from phidippus spider venom; John Randolph Hunter Jackson, et al., 514/12; 530/350 [IMAGE AVAILABLE]

51. 5,753,258, May 19, 1998, Artificial viral envelopes; Hans Schreier, et al., 424/450, 130.1, 184.1, 188.1; 436/829 [IMAGE AVAILABLE]

52. 5,741,669, Apr. 21, 1998, Insecticidally effective peptides; Karen Joanne Krapcho, et al., 435/69.1; 424/405; 435/70.1, 320.1; 514/12; 536/23.5 [IMAGE AVAILABLE]

53. 5,736,131, Apr. 7, 1998, Hybrid toxin; Hendrik Jan Bosch, et al., 800/300; 424/93.1, 93.2, 93.461; 435/69.7, 252.3, 252.31, 254.11, 320.1; 514/2, 12; 530/350; 536/23.4, 23.71 [IMAGE AVAILABLE]

54. 5,723,756, Mar. 3, 1998, Bacillus thuringiensis strains and their genes encoding insecticidal toxins; Marnix Peferoen, et al., 800/279; 435/69.1, 410; 536/23.71; 800/294, 301, 317.2 [IMAGE AVAILABLE]

55. 5,710,020, Jan. 20, 1998, Bacillus *thuringiensis* .alpha.-*endotoxin* fragments; Michael J. Adang, 435/69.1, 252.31, 252.33; 536/23.71 [IMAGE AVAILABLE]

56. 5,695,999, Dec. 9, 1997, Regeneration of cotton plant in suspension culture; Thirumale S. Rangan, et al., 435/427, 430.1, 431 [IMAGE AVAILABLE]

57. 5,695,959, Dec. 9, 1997, Recombinant expression of insecticidally effective spider toxin; John Randolph Hunter Jackson, et al., 435/69.1, 252.3, 320.1, 325, 348; 514/44 [IMAGE AVAILABLE]

58. 5,689,044, Nov. 18, 1997, Chemically inducible promoter of a plant PR-1 gene; John A. Ryals, et al., 800/301; 435/320.1, 418, 419; 536/23.6, 24.1; 800/300, 302 [IMAGE AVAILABLE]

59. 5,688,764, Nov. 18, 1997, Insecticidal peptides from spider venom; Janice H. Johnson, et al., 514/12; 424/93.2; 435/69.1, 252.3, 254.11, 320.1, 348; 514/2; 530/300, 858; 536/23.5 [IMAGE AVAILABLE]

60. 5,674,846, Oct. 7, 1997, Insecticidal peptides from Segestria sp. spider venom; Janice H. Johnson, et al., 514/12; 530/300, 350, 858 [IMAGE AVAILABLE]

61. 5,659,124, Aug. 19, 1997, Transgenic male sterile plants for the production of hybrid seeds; Lyle D. Crossland, et al., 800/267; 47/DIG.1; 435/69.1, 70.1; 536/23.6, 23.72, 24.1, 24.5; 800/268, 274, 287, 303 [IMAGE AVAILABLE]

62. 5,659,123, Aug. 19, 1997, Diabrotica toxins; Jeroen Van Rie, et al., 800/302; 514/12; 536/23.71; 800/320.1 [IMAGE AVAILABLE]

63. 5,658,781, Aug. 19, 1997, Insecticidally effective peptides; Karen J. Krapcho, et al., 435/6, 252.3, 320.1, 325, 348, 349, 418; 536/23.5, 24.3 [IMAGE AVAILABLE]

64. 5,658,563, Aug. 19, 1997, Insecticidally effective peptides; Karen J. Krapcho, et al., 424/93.2; 435/320.1 [IMAGE AVAILABLE]

65. 5,654,414, Aug. 5, 1997, Chemically inducible promoter of a cucumber chitinase/lysozyme gene; John A. Ryals, et al., 800/279; 435/69.1, 200, 206, 3[illegible] 536/23.6; 800/317.3 [IMAGE AVAILABLE]

6 6. 5,650,505, Jul. 22, 1997, Chemically regulatable and anti-pathogenic DNA sequences and uses thereof; John A. Ryals, et al., 800/301; 435/69.1, 320.1, 418, 419; 530/370, 379; 536/23.6, 24.5; 800/317.3 [IMAGE AVAILABLE]

67. 5,650,308, Jul. 22, 1997, Recombinant Bacillus thuringiensis strain construction method; James A. Baum, 435/485, 252.31, 320.1 [IMAGE AVAILABLE]

68. 5,625,136, Apr. 29, 1997, Synthetic DNA sequence having enhanced insecticidal activity in maize; Michael G. Koziel, et al., 800/302; 435/69.1; 536/23.1, 23.71 [IMAGE AVAILABLE]

69. 5,614,395, Mar. 25, 1997, Chemically regulatable and anti-pathogenic DNA sequences and uses thereof; John A. Ryals, et al., 435/6, 4, 69.1, 468; 536/24.1; 800/279 [IMAGE AVAILABLE]

70. 5,608,142, Mar. 4, 1997, Insecticidal cotton plants; Kenneth A. Barton, et al., 800/302; 435/320.1 [IMAGE AVAILABLE]

71. 5,595,733, Jan. 21, 1997, Methods for protecting ZEA mays plants against pest damage; Gleta Carswell, et al., 424/93.21; 536/23.71; 800/302, 320.1 [IMAGE AVAILABLE]

72. 5,593,881, Jan. 14, 1997, Bacillus *thuringiensis* delta-*endotoxin*; Mark Thompson, et al., 435/418, 252.3, 320.1; 536/23.71 [IMAGE AVAILABLE]

73. 5,593,874, Jan. 14, 1997, Enhanced expression in plants; Sherri M. Brown, et al., 800/279; 435/69.1; 536/24.1; 800/300, 300.1, 301, 302, 320.1, 320.2, 320.3 [IMAGE AVAILABLE]

74. 5,583,036, Dec. 10, 1996, Regeneration of cotton plant in suspension culture; Thirumale S. Rangan, et al., 435/427 [IMAGE AVAILABLE]

75. 5,567,862, Oct. 22, 1996, Synthetic insecticidal crystal protein gene; Michael J. Adang, et al., 800/302; 435/69.1, 418, 468 [IMAGE AVAILABLE]

76. 5,567,600, Oct. 22, 1996, Synthetic insecticidal crystal protein gene; Michael J. Adang, et al., 536/23.71; 435/69.1, 418, 468, 469, 470; 800/279 [IMAGE AVAILABLE]

77. 5,554,798, Sep. 10, 1996, Fertile glyphosate-resistant transgenic corn plants; Ronald C. Lundquist, et al., 800/300.1; 536/23.71 [IMAGE AVAILABLE]

78. 5,547,871, Aug. 20, 1996, Heterologous signal sequences for secretion of insect controlling proteins; Bruce C. Black, et al., 435/348, 69.8, 320.1; 536/23.2, 23.4, 23.51, 23.71 [IMAGE AVAILABLE]

79. 5,545,565, Aug. 13, 1996, Transformation vectors allowing expression of foreign polypeptide endoxins from Bacillus thuringiensis in plants; Henri M. J. De Greve, et al., 435/320.1, 69.1; 514/12; 536/23.71 [IMAGE AVAILABLE]

80. 5,538,880, Jul. 23, 1996, Method for preparing fertile transgenic corn plants; Ronald C. Lundquist, et al., 800/265; 435/430.1; 800/275, 279, 293 [IMAGE AVAILABLE]

81. 5,538,877, Jul. 23, 1996, Method for preparing fertile transgenic corn plants; Ronald C. Lundquist, et al., 800/265; 435/424; 800/268, 275, 279, 293 [IMAGE AVAILABLE]

82. 5,530,195, Jun. 25, 1996, Bacillus *thuringiensis* gene encoding a *toxin* active against insects; Vance C. Kramer, et al., 800/302; 424/93.2; 435/69.1, 235.1, 252.3, 252.31, 252.34, 320.1; 514/12; 530/350; 536/23.71 [IMAGE AVAILABLE]

83. 5,527,883, Jun. 18, 1996, Delta-endotoxin expression in pseudomonas fluorescens; Mark Thompson, et al., 530/350; 435/252.34, 320.1; 536/23.4, 23.71 [IMAGE AVAILABLE]

84. 5,516,693, May 14, 1996, Hybrid gene incorporating a DNA fragment containing a gene coding for an insecticidal protein, plasmids, transformed cyanobacteria expressing such protein and method for use as a biocontrol agent; Mark A. Vaeck, et al., 435/320.1, 69.7, 252.33; 536/23.4, 23.71 [IMAGE AVAILABLE]

85. 5,508,468, Apr. 16, 1996, Fertile transgenic corn plants; Ronald C. Lundquist, et al., 800/300.1, 301, 302, 320.1 [IMAGE AVAILABLE]

86. 5,508,264, Apr. 16, 1996, Pesticidal compositions; Gregory A. Bradfisch, et al., 514/12; 530/350 [IMAGE AVAILABLE]

87. 5,500,365, Mar. 19, 1996, Synthetic plant genes; David A. Fischhoff, et al., 435/418, 411, 414, 417; 536/23.71 [IMAGE AVAILABLE]

88. 5,495,071, Feb. 27, 1996, Insect resistant tomato and potato plants; David A. Fischhoff, et al., 800/302; 435/69.1, 320.1, 411, 417, 418; 514/12; 536/23.71 [IMAGE AVAILABLE]

89. 5,484,956, Jan. 16, 1996, Fertile transgenic Zea mays plant comprising heterologous DNA encoding Bacillus *thuringiensis* *endotoxin*; Ronald C. Lundquist, et al., 800/302; 536/23.71 [IMAGE AVAILABLE]

90. 5,477,002, Dec. 19, 1995, Anther-specific CDNA sequences, genomic DNA sequences and recumbinant DNA sequences; Annmarie B. Tuttle, et al., 800/303; 435/320.1; 536/23.1, 23.5, 23.6, 23.7, 24.1; 800/317.3 [IMAGE AVAILABLE]

91. 5,466,785, Nov. 14, 1995, Tissue-preferential promoters; Annick J. de Framond, 536/24.1; 424/93.2; 435/320.1; 536/23.7 [IMAGE AVAILABLE]

92. 5,461,032, Oct. 24, 1995, Insecticidally effective peptides; Karen J. Krapcho, et al., 514/12; 435/69.1 [IMAGE AVAILABLE]

93. 5,460,963, Oct. 24, 1995, Plants transformed with a DNA sequence from Bacillus thuringiensis lethal to Lepidoptera; Johan Botterman, et al., 800/279; 435/71.3, 320.1, 411, 414, 418; 530/350; 536/23.71 [IMAGE AVAILABLE]

94. 5,457,178, Oct. 10, 1995, Insecticidally effective spider toxin; John R. H. Jackson, et al., 530/350 [IMAGE AVAILABLE]

95. 5,441,934, Aug. 15, 1995, Insecticidally effective peptides; Karen J. Krapcho, et al., 514/12; 424/405, 538; 435/69.1; 530/300, 324, 345 [IMAGE AVAILABLE]

96. 5,441,884, Aug. 15, 1995, Bacillus thuringiensis transposon TN5401; James A. Baum, 435/252.31; 424/93.2; 435/252.3, 252.33, 320.1; 536/23.1, 23.2, 23.7, 24.1 [IMAGE AVAILABLE]

97. 5,424,412, Jun. 13, 1995, Enhanced expression in plants; Sherri M. Brown, et al., 536/24.1; 435/69.1, 70.1, 320.1 [IMAGE AVAILABLE]

98. 5,424,409, Jun. 13, 1995, DNA constructs encoding Bacillus thuringiensis toxins from strain A20; Susan Ely, et al., 536/23.71; 424/93.461; 536/23.4 [IMAGE AVAILABLE]

99. 5,422,120, Jun. 6, 1995, Heterovesicular liposomes; Sinil Kim, 424/450; 264/4.1, 4.3, 4.6; 436/829 [IMAGE AVAILABLE]

100. 5,409,823, Apr. 25, 1995, Methods for the production of hybrid seed; Lyle D. Crossland, et al., 800/274; 47/DIG.1; 536/24.1; 800/287, 303 [IMAGE AVAILABLE]

101. 5,380,831, Jan. 10, 1995, Synthetic insecticidal crystal protein gene; Michael J. Adang, et al., 536/23.71; 435/69.1, 91.1, 91.5, 91.52 [IMAGE AVAILABLE]

102. 5,371,003, Dec. 6, 1994, Electrotransformation process; Lynn E. Murry, et al., 800/292; 435/6, 173.5, 173.6, 470 [IMAGE AVAILABLE]

103. 5,350,689, Sep. 27, 1994, Zea mays plants and transgenic Zea mays plants regenerated from protoplasts or protoplast-derived cells; Ray Shillito, et al., 435/412, 421 [IMAGE AVAILABLE]

104. 5,349,124, Sep. 20, 1994, Insect-resistant lettuce plants; David A. Fischhoff, et al., 800/302; 424/93.21; 435/418 [IMAGE AVAILABLE]

105. 5,338,544, Aug. 16, 1994, CryIIB protein, insecticidal compositions and methods of use thereof; William P. Donovan, 424/93.2, 93.461; 435/69.1, 252.31; 514/2; 530/350 [IMAGE AVAILABLE]

106. 5,317,096, May 31, 1994, Transformation vectors allowing expression of foreign polypeptide endotoxins from Bacillus thuringiensis in plants; Henri M. J. De Greve, et al., 536/23.71 [IMAGE AVAILABLE]

107. 5,306,628, Apr. 26, 1994, Method and means for extending the host range of insecticidal proteins; Natarajan Sivasubramanian, et al., 435/69.7, 320.1; 530/350; 536/23.71 [IMAGE AVAILABLE]

108. 5,290,914, Mar. 1, 1994, Hybrid diphtheria-B.t. pesticidal toxins; Edward Wilcox, et al., 530/350; 435/69.7; 514/2, 12 [IMAGE AVAILABLE]

109. 5,281,532, Jan. 25, 1994, Pseudomas hosts transformed with bacillus endotoxin genes; David H. Rammler, et al., 435/252.34; 424/93.2; 435/69.1, 320.1; 536/23.71 [IMAGE AVAILABLE]

110. 5,254,799, Oct. 19, 1993, Transformation vectors allowing expression of Bacillus thuringiensis endotoxins in plants; Henri M. J. De Greve, et al., 800/302; 435/418 [IMAGE AVAILABLE]

111. 5,250,515, Oct. 5, 1993, Method for improving the efficacy of insect toxins; Roy L. Fuchs, et al., 514/12; 424/93.461, 195.1; 530/370, 379 [IMAGE AVAILABLE]

112. 5,244,802, Sep. 14, 1993, Regeneration of cotton; Thirumale S. Rangan, 435/427; 47/58.1 [IMAGE AVAILABLE]

113. 5,229,113, Jul. 20, 1993, Bradyrhizobium japonicum nodulation inducing factor; Renee Kosslak, et al., 424/93.2; 47/57.6, DIG.9, DIG.10; 71/7; 435/252.2, 878; 504/117 [IMAGE AVAILABLE]

114. 5,177,308, Jan. 5, 1993, Insecticidal toxins in plants; Kenneth A. Barton, et al., 800/302; 435/320.1 [IMAGE AVAILABLE]

115. 5,143,905, Sep. 1, 1992, Method and means for extending the host range of insecticidal proteins; Natarajan Sivasubramanian, et al., 514/21; 424/405; 435/69.7; 514/8, 12; 530/350, 409 [IMAGE AVAILABLE]

116. 5,141,745, Aug. 25, 1992, Nodulation inducing factors; Barry G. Rolfe, et al., 424/93.4; 47/57.6, DIG.9, DIG.10; 71/7; 435/252.2, 878; 504/117, 292 [IMAGE AVAILABLE]

117. 5,133,962, Jul. 28, 1992, Method of controlling coleopteran insects with Bacillus thuringiensis; August J. Sick, et al., 424/93.2; 435/69.1, 71.2, 252.3, 822, 823, 829, 831, 847, 874, 880, 911, 946; 536/23.71 [IMAGE AVAILABLE]

118. 5,128,130, Jul. 7, 1992, Hybrid Bacillus thuringiensis gene, plasmid and transformed Pseudomonas fluorescens; Thomas E. Gilroy, et al., 424/93.2; 435/69.1, 71.2, 91.41, 170, 252.3, 320.1, 832, 848, 874; 530/350; 536/23.71 [IMAGE AVAILABLE]

119. 5,110,905, May 5, 1992, Activated Bacillus thuringienses delta-endotoxin produced by an engineered hybrid gene; Daniel P. Witt, et al., 530/350; 435/69.1, 71.1 [IMAGE AVAILABLE]

120. 5,104,974, Apr. 14, 1992, Bacillus *thuringiensis* coleopteran-active *toxin*; August J. Sick, et al., 530/350; 435/69.1, 71.1, 252.3, 254.2, 254.21, 320.1, 822, 911, 946; 530/825; 536/23.71 [IMAGE AVAILABLE]

121. 5,073,632, Dec. 17, 1991, CryIIB crystal protein gene from Bacillus thuringiensis; William P. Donovan, 536/23.71, 24.1 [IMAGE AVAILABLE]

122. 5,055,294, Oct. 8, 1991, *Chimeric* Bacillus *thuringiensis* crystal protein gene comprising HD-73 and Berliner 1715 *toxin* genes, transformed and expressed in Pseudomonas fluorescens; Thomas E. Gilroy, 424/93.2, 93.21; 435/69.1, 69.7, 252.3, 252.31, 252.32, 252.33, 252.34, 254.11, 254.2, 320.1; 536/23.71 [IMAGE AVAILABLE]

123. 5,015,580, May 14, 1991, Particle-mediated transformation of soybean plants and lines; Paul Christou, et al., 800/267; 435/317.1, 320.1; 800/268, 293 [IMAGE AVAILABLE]

124. 5,010,001, Apr. 23, 1991, Preparation of natural or modified insect toxins; Thomas J. Pollock, 435/69.1, 252.33, 320.1; 536/23.71 [IMAGE AVAILABLE]

125. 4,996,155, Feb. 26, 1991, Bacillus *thuringiensis* gene encoding a coleopteran-active *toxin*; August J. Sick, et al., 424/93.2, 93.21; 435/69.1, 71.1, 252.3, 252.5, 254.11, 254.2, 254.21, 320.1, 822, 911, 946; 536/23.71, 24.2 [IMAGE AVAILABLE]

126. H 875, Jan. 1, 1991, *Toxin*-encoding nucleic acid fragments derived from a Bacillus *thuringiensis* subsp. israelensis gene; David J. Ellar, et al., 435/252.31, 69.1, 252.5, 832; 530/350, 858; 536/23.7, 23.71 [IMAGE AVAILABLE]

127. 4,945,057, Jul. 31, 1990, Monoclonal antibodies to crystal protein of Bacillus thuringiensis subspecies israelensis; Kevin B. Temeyer, et al., 530/388.4; 435/70.21, 340, 832, 948; 436/548; 530/809, 825 [IMAGE AVAILABLE]

128. 4,695,455, Sep. 22, 1987, Cellular encapsulation of pesticides produced by expression of heterologous genes; Andrew C. Barnes, et al., 424/93.2, 93.21, 520; 435/69.1, 252.3, 252.31, 252.33, 252.34, 254.11, 254.2, 254.21, 260, 317.1; 514/2 [IMAGE AVAILABLE]

US PAT NO: 5,874,288 [IMAGE AVAILABLE] L6: 10 of 128

BSUM(15) The soil microbe Bacillus *thuringiensis* (B.t.) is a Gram-positive, spore-forming bacterium characterized by parasporal crystalline protein inclusions. These inclusions often appear microscopically as distinctively shaped crystals. The proteins can be highly toxic to pests and specific in their toxic activity. Certain B.t. *toxin* genes have been isolated and sequenced, and recombinant DNA-based B.t. products have been produced and approved for use. In addition,. . . approaches for delivering these B.t. endotoxins to agricultural environments are under development, including the use of plants genetically engineered with *endotoxin* genes for insect resistance and the use of stabilized intact microbial cells as B.t. *endotoxin* delivery vehicles (Gaertner, F. H., L. Kim [1988] TIBTECH6:S4-S7). Thus, isolated B.t. *endotoxin* genes are becoming commercially valuable.

BSUM(16) Until . . . has been largely restricted to a narrow range of lepidopteran (caterpillar) pests. Preparations of the spores and crystals of B. *thuringiensis* subsp. kurstaki have been used for many years as commercial insecticides for lepidopteran pests. For example, B. *thuringiensis* var. kurstaki HD-1 produces a crystalline .delta.-*endotoxin* which is toxic to the larvae of a number of lepidopteran insects.

BSUM(19) The . . . and Whiteley for crystal proteins was based on both the deduced amino acid sequence and the host range of the *toxin*. That system was adapted to cover 14 different types of *toxin* genes which were divided into five major classes. As more *toxin* genes were discovered, that system started to become unworkable, as genes with similar sequences were found to have significantly different insecticidal specificities. The number of sequenced Bacillus *thuringiensis* crystal protein genes currently stands at about 50. A revised nomenclature scheme has beenproposed which is based solely on amino acid identity (Crickmore et al. [1996] Society for Invertebrate Pathology, 29th Annual Meeting. IIIrd International Colloquium on Bacillus *thuringiensis*, University of Cordoba, Cordoba, Spain, September.1-6, abstract). The mnemonic "cry" has been retained for all of the *toxin* genes except cyta and cytB, which remain a separate class. Roman numerals have been exchanged for Arabic numerals in the. . .

BSUM(21) A small number of research articles have been published about the effects of delta endotoxins from B. *thuringiensis* species on the viability of nematode eggs. Bottjer, Bone and Gill, ([1985] Experimental Parasitology 60:239-244)have reported that B.t. kurstaki and. . . were tested with widely variable toxicities. Ignoffo and Dropkin ([1977] J Kans. Entomol. Soc. 50:394-398) have reported that the thermostable *toxin* from Bacillus *thuringiensis* (beta exotoxin) was active against a free-living nematode, Panagrellus redivivus (Goodey); a plant-parasitic nematode, Meloidogyne incognita (Chitwood); and a fungus-feeding. . . specificity. Also, Ciordia and Bizzell ([1961] Jour. of Parasitology 47:41 [abstract]) gave a preliminary report on the effects of B. *thuringiensis* on some cattle nematodes.

BSUM(23) Some Bacillus *thuringiensis* toxins which are active against corn rootworm and other coleopterans are now known. For example, U.S. Pat. No. 4,849,217 discloses. . . Isolates and toxins active against coleopterans. Specifically disclosed in these patents is the isolate known as PS86A1 and a coleopteran-active *toxin* obtainable therefrom known as 86A1. "Toxin" 86A1 is now also known as Cry6A (CryVIA). The wild-type Cry6A *toxin* is about 54-58 kDa.

DETD(15) The B.t. PS86A1 isolate produces an approximately 55 kDa *toxin* is referred to as the 86A1 or 86AI (a) *toxin*. This *toxin* is a Cry6A *toxin*. The gene encoding this *toxin* has been cloned into Bacillus *thuringiensis* isolate MR506, which also expresses the Cry6A *toxin*.

DETD(21) One recombinant host which can be used to obtain the truncated *toxin* of the subject invention is MR506. The truncated *toxin* of the subject invention can be obtained by treating the crystalline .delta.-*endotoxin* of Bacillus *thuringiensis* strain MR506 with a serine protease such as bovine trypsin at an alkaline pH and preferably in the absence of. . .

DETD(92) A *fusion* protein consisting of Cry6B and Cry6A having activity against western corn rootworm can be constructed. It should be noted that. . . Cry6B/69D1 protein was not previously known to be useful for controlling corn rootworm. The sequence of the fall length Cry6B *toxin* obtainable from PS69D1 corresponds to SEQ ID NO.10. See also SEQ ID NO. 9.

We claim:

1. A polynucleotide sequence which encodes a Bacillus *thuringiensis* Cry6A *toxin* for controlling coleopterans, wherein said *toxin* is truncated compared to the full length *toxin* as it is naturally expressed, wherein said *toxin* has the amino acid sequence of SEQ ID NO. 6.

US PAT NO: 5,872,212 [IMAGE AVAILABLE] L6: 11 of 128

DETD(38) One strategy of altering pesticidal or auxiliary proteins is to *fuse* a 15-amino-acid "S-tag" to the protein without destroying the insect cell binding domain(s), translocation domains or protein-protein interacting domains of. . . H. W. Wyckoff (1971) in "The Enzymes", Vol. IV (Boyer, P. D. ed.). pp. 647-805. Academic Press, New York). The *fusion* can be made in such a way as to destroy or remove the cytotoxic activity of the pesticidal or auxiliary protein, thereby replacing the VIP cytotoxic activity with a new cytotoxic ribonuclease activity. The final *toxin* would be comprised of the S-protein, a pesticidal protein and an auxiliary protein, where either the pesticidal protein or the auxiliary protein is produced as translational *fusions* with the S-tag. Similar strategies can be used to *fuse* other potential cytotoxins to pesticidal or auxiliary proteins including (but not limited to) ribosome inactivating proteins, insect hormones, hormone receptors,. . .

DETD(82)Various strains of Bacillus *thuringiensis* are used in this manner. Such Bt strains produce *endotoxin* protein(s) as well as VIPs. Alternatively, such strains can produce only VIPs. A sporulation deficient strain of Bacillus subtilis has been shown to produce high levels of the CryIIIA *endotoxin* from Bacillus *thuringiensis* (Agaisse, H. and Lereclus, D., "Expression in Bacillus subtilis of the Bacillus *thuringiensis* CryIIIA *toxin* gene is not dependent on a sporulation-specific sigma factor and is increased in a spoOA mutant", J. Bacteriol., 176:4734-4741 (1994)). A similar spoOA mutant can be prepared in Bacillus *thuringiensis* and used to produce encapsulated VIPs which are not secreted into the medium but are retained within the cell.

DETD(93) An . . . 3-9 of the NH.sub.2 -terminus has been generated. The probe was synthesized based on the codon usage of a Bacillus *thuringiensis* (Bt) .delta.-*endotoxin* gene. The nucleotide sequence of the oligonucleotide probe used for Southern hybridizations was as follows:

DETD(151) An . . . of the N-terminal sequence (Example 5) was generated. The probe was synthesized based on the codon usage of a Bacillus *thuringiensis* (Bt) .delta.-*endotoxin* gene. The nucleotide sequence

DETD(296) To . . . 96 well microtiter dishes (five plates total) until the cultures sporulated. Of the strains tested, 288 were categorized as Bacillus *thuringiensis*, and 175 were categorized as other Bacillus species based on the presence or absence of .delta.-*endotoxin* crystals. For each microtiter dish, a 96-pin colony stamper was used to transfer approximately 10 .mu.l of spore culture to. . .

DETD(317) One example of a *fusion* construction comprising a maize optimized DNA sequence encoding a single polypeptide chain *fusion* having VIP2A(a) at the N-terminal end and VIP1A(a) at the C-terminal end is provided by pCIB553 1. A DNA sequence. . . codon in VIP2A(a) was removed using PCR and replaced by the BglII restriction site with a SmaI site. A translation *fusion* was made by ligating the Bam HI/PstI fragment of the VIP2A(a) gene from pCIB5522 (see Example 24), a PCR fragment. . . BamHI at the 3' end and the modified synthetic VIP1A(a) gene from pCIB5526 described below (See SEQ ID NO:35). The *fusion* was obtained by a four way ligation that resulted in a plasmid containing the VIP2A(a) gene without a translation stop. . . without the Bacillus secretion signal. The DNA sequence for this construction is disclosed in SEQ ID NO:49, which encodes the *fusion* protein disclosed in SEQ ID NO:50. A single polypeptide *fusion* where VIP1A(a) is at the N-terminal end and VIP2A(a) is at the C-terminal end can be made in a similar fashion. Furthermore, either one or both genes can be linked in a translation *fusion* with or without a linker at either the 5' or the 3' end to other molecules like *toxin* encoding genes or reporter genes.

US PAT NO: 5,827,514 [IMAGE AVAILABLE] L6: 31 of 128

ABSTRACT: Disclosed are compositions and processes for controlling lepidopteran pests. These compositions comprise synergistic combinations of a CryIF *chimeric* and CryIA(c) *chimeric* Bacillus *thuringiensis* .delta.-*endotoxin*. These compositions have been found to exhibit excellent activity against lepidopteran pests.

BSUM(2) The soil microbe Bacillus *thuringiensis* (B.t.) is a Gram-positive, spore-forming bacterium characterized by parasporal crystalline protein inclusions. These inclusions often appear microscopically as distinctively shaped crystals. The proteins can be highly toxic to pests and specific in their toxic activity. Certain B.t. *toxin* genes have been isolated and sequenced, and recombinant DNA-based B.t. products have been produced and approved for use. In addition,. . . approaches for delivering these B.t. endotoxins to agricultural environments are under development, including the use of plants genetically engineered with *endotoxin* genes for insect resistance and the use of stabilized intact microbial cells as B.t. *endotoxin* delivery vehicles (Gaertner, F. H., L. Kim [1988] TIBTECH 6:S4-S7). Thus, isolated B.t. *endotoxin* genes are becoming commercially valuable.

BSUM(3) Until . . . has been largely restricted to a narrow range of lepidopteran (caterpillar) pests. Preparations of the spores and crystals of B. *thuringiensis* subsp. kurstaki have been used for many years as commercial insecticides for lepidopteran pests. For example, B. *thuringiensis* var. kurstaki HD-1 produces a crystal called a .delta.-*endotoxin* which is toxic to the larvae of a number of lepidopteran insects.

BSUM(8) A majority of Bacillus *thuringiensis* .delta.-*endotoxin* crystal protein molecules are composed of two functional segments. The protease-resistant core *toxin* is the first segment and corresponds to about the first half of the protein molecule. The three-dimensional structure of a core segment of a cryIIIA B.t. .delta.-*endotoxin* is known and it is proposed that all related toxins have that same overall structure (Li, J., J. Carroll, D.. . . this second segment will be referred to herein as the *protoxin* segment." The protoxin segment is believed to participate in *toxin* crystal formation (Arvidson, H., P. E. Dunn, S. Strand, A. I. Aronson [1989] Molecular Microbiology 3:1533-1534; Choma, C. T., W.. . . K. Surewicz, P. R. Carey, M. Pozsgay, T. Raynor, H. Kaplan [1990] Eur. J. Biochem. 189:523-527). The full 130 kDa *toxin* molecule is rapidly processed to the resistant core segment by protease in the insect gut. The protoxin segment may thus convey a partial insect specificity for the *toxin* by limiting the accessibility of the core to the insect by reducing the protease processing of the *toxin* molecule (Haider, M. Z., B. H. Knowles, D. J. Ellar [1986] Eur. J. Biochem. 156:531-540) or by reducing *toxin* solubility (Aronson, A. I., E. S. Han, W. McGaughey, D. Johnson [1991] Appl. Environ. Microbiol. 57:981-986).

BSUM(9) *Chimeric* proteins joined within the *toxin* domains have been reported between CryIC and CryIA(b) (Honee, G., D. Convents, J. Van Rie, S. Jansens, M. Perferoen, B. Visser [1991] Mol. Microbiol. 5:2799-2806); however, the activity of these *chimeric* proteins was either much less, or undetectable, when compared to CryIC on a relevant insect.

BSUM(10) Honee et al. (Honee, G., W. Vriezen, B. Visser [1990] Appl. Environ. Microbiol. 56:823-825) also reported making a *chimeric* *fusion* protein by linking tandem *toxin* domains of CryIC and CryIA(b). The resulting protein had an increased spectrum of activity equivalent to the combined activities of the individual toxins; however, the activity of the *chimeric* was not increased toward any one of the target insects.

BSUM(14) The subject invention concerns the discovery of advantageous increased activity against lepidopteran pests achieved by the combination of two Bacillus *thuringiensis* (B.t.) .delta.-*endotoxin* proteins. More specifically, a CryIF *chimeric* *toxin* combined with a CryIA(c) *chimeric* *toxin* act in synergy to yield unexpected enhanced toxicity to lepidopteran pests.

BSUM(16) *Chimeric* CryIF genes useful according to the subject invention can be assembled that substitute a heterologous protoxin segment for all or. . . can be used in place of all or part of the region which encodes the protoxin for a native cryIF *toxin*. Similarly, a *chimeric* gene can be constructed wherein the region encoding all or part of the protoxin of a cryIF *toxin* is replaced by DNA encoding all or part of the protoxin of a cryIA(c)/cryIA(b) *chimeric* gene. In a specific embodiment, the cryIA(c)/cryIA(b) *chimeric* gene is that which has been denoted 436 and which is described in U.S. Pat. No. 5,128,130. This gene can. . .

DRAWING DESC: FIG. 4 The NsiI *toxin*-containing fragment with the new restriction sites is ligated to the vector-containing DNA from pMYC1050.DELTA.BamHI to give pMYC2244. A BamHI-PvuI PCR-derived DNA fragment containing the cryIF *toxin* is exchanged for the equivalent fragment in pMYC2244. The resulting *chimera* is called pMYC2239. B=BamHI, C=ClaI, H=HindIII, N=NsiI, P=PvuI.

DRAWING DESC: FIG. . . . The small ApaI DNA fragment of pMYC2047 is substituted for the homologous region of pMYC2239 to give plasmid pMYC2244. This "chimera" consists of cryIF in the *toxin* region and crylA(b) in the protoxin. C=ClaI, H=HindIII, N=NsiI, P=PvuI.

DRAWING DESC: FIG. 8 A *chimeric* *toxin* containing the 436 protoxin is constructed by substituting a PCR-generated PvuI-BstEII protoxin DNA for the homologous fragment in pMYC2523. The. . .

DETD(23) SEQ ID NO. 22 shows the *toxin*-encoding DNA sequence of pMYC2244, which encodes a cryIF/crylA(b) *chimeric* *toxin*.

DETD(24) SEQ ID NO. 23 shows the predicted amino acid sequence of the cryIF/crylA(b) *chimeric* *toxin* encoded by pMYC2244.

DETD(27) SEQ ID NO. 26 shows the *toxin*-encoding DNA sequence of pMYC2523, which encodes a cryIF/crylA(b) *chimeric* *toxin* with codon rework.

DETD(29) SEQ ID NO. 28 shows the *toxin*-encoding DNA sequence of pMYC2254, which encodes a cryIF/436 *chimeric* *toxin*.

DETD(36) SEQ ID NO. 35 shows the amino acid sequence of a CryIF/CryIA(b) *chimeric* *toxin* of the subject invention that corresponds to the "Cons" sequence shown in FIG. 9.

DETD(37) SEQ ID NO. 36 shows the amino acid sequence of a *chimeric* *toxin* of the subject invention that incorporates the alternative amino acids as shown in the first "Alt" sequence listed above the. . .

DETD(38) SEQ ID NO. 37 shows the amino acid sequence of a *chimeric* *toxin* of the subject invention that incorporates the alternative amino acids as shown in the second "Alt" sequence listed above the. . .

DETD(39) SEQ ID NO. 38 shows the amino acid sequence of a *chimeric* *toxin* of the subject invention that incorporates the alternative amino acids as shown in the third "Alt" sequence listed above the. . .

DETD(41) The subject invention concerns the unexpected enhanced pesticidal activity resulting from the combination of a CryIF *chimeric* *toxin* and a CryIA(c) *chimeric* *toxin*. The combination surprisingly has increased activity against lepidopteran pests. Preparations of combinations of isolates that produce the two *chimeric* toxins can be used to practice the subject invention. Pseudomonas fluorescens cells transformed with B.t. genes can serve as one. . . of the toxins of the subject invention. For example, a lactose-inducible P. fluorescens strain comprising a gene encoding a CryIF/CryIA(b) *toxin*, and P. fluorescens MR436, which

comprises a gene encoding a CrylA(c)/CrylA(b) "chimeric" "toxin", can be used to practice the subject invention. These two Pseudomonas strains can be combined in a physical blend that. . .

DETD(45) In accordance with the subject invention, it has been discovered that products comprising the two "chimeric" toxins have been discovered to require a lower total protein content for product application, thus providing the user greater economy. Insects which are less susceptible to the action of a single "toxin" will be more greatly affected by the combination of toxins of the subject invention, rendering a product containing the two toxins more efficacious than products containing a single "toxin". Additionally, pests are less likely to develop a rapid resistance to a product containing the two toxins, than to products containing a single "toxin".

DETD(47) The "chimeric" toxins of the subject invention comprise a full core N-terminal "toxin" portion of a B.t. "toxin" and, at some point past the end of the "toxin" portion, the protein has a transition to a heterologous protoxin sequence. The N-terminal "toxin" portion of a B.t. "toxin" is refererred to herein as the "core" "toxin". The transition to the heterologous protoxin segment can occur at approximately the "toxin"/protoxin junction or, in the alternative, a portion of the native protoxin (extending past the "toxin" portion) can be retained with the transition to the heterologous protoxin occurring downstream. As an example, one "chimeric" "toxin" of the subject invention has the full "toxin" portion of cryIF (amino acids 1-601) and a heterologous protoxin (amino acids 602 to the C-terminus). In a preferred embodiment, the heterologous portion of the protoxin is derived from a crylA(b) or 436 "toxin".

DETD(48) A . . . certain class such as cryIF, will vary to some extent in length and the precise location of the transition from "toxin" portion to protoxin portion. Typically, the crylA(b) and cryIF toxins are about 1150 to about 1200 amino acids in length. The transition from "toxin" portion to protoxin portion will typically occur at between about 50% to about 60% of the full length "toxin". The "chimeric" "toxin" of the subject invention will include the full expanse of this core N-terminal "toxin" portion. Thus, the "chimeric" "toxin" will comprise at least about 50% of the full length cryIF B.t. "toxin". This will typically be at least about 590 amino acids. With regard to the protoxin portion, the full expanse of the crylA(b) protoxin portion extends from the end of the "toxin" portion to the C-terminus of the molecule. It is the last about 100 to 150 amino acids of this portion which are most critical to include in the "chimeric" "toxin" of the subject invention. In a "chimeric" "toxin" specifically exemplified herein, at least amino acids 1043 (of SEQ ID NO. 23) to the C-terminus of the crylA(b) molecule. . . marks the location in the protoxin segment of the molecule beyond which heterologous amino acids will always occur in the "chimeric" "toxin". In another example, the peptide shown as SEQ ID NO. 31 occurs at amino acids 1061 to 1068. In this. . . approximately 5 to 10% of the overall B.t. protein which should comprise heterologous DNA (compared to the cryIF core N-terminal "toxin" portion) in the "chimeric" "toxin" of the subject invention. In the specific examples contained herein, heterologous protoxin sequences occur from amino acid 640 to the. . .

DETD(49) Thus, a preferred embodiment of the subject invention is a "chimeric" B.t. "toxin" of about 1150 to about 1200 amino acids in length, wherein the "chimeric" "toxin" comprises a cryIF core N-terminal "toxin" portion of at least about 50 to 60% of a full cryIF molecule, but no more than about 90 to 95% of the full molecule. The "chimeric" "toxin" further comprises a crylA(b) or a 436 protoxin C-terminal portion which comprises at least about 5 to 10% of the. . . transition from cryIF to crylA(b) or 436 sequence thus occurs within the protoxin segment (or at the junction of the "toxin" and protoxin segments) between about 50% and about 95% of the way through the molecule. In the specific examples provided. . .

DETD(50) A specific embodiment of the subject invention is the "chimeric" "toxin" shown in FIG. 9. Other constructs may be made and used by those skilled in this art having the benefit of the teachings provided herein. The core "toxin" segment of cryI proteins characteristically ends with the sequence: Val/Leu Tyr/Ile Ile Asp Arg/Lys Ile/Phe Glu Ile/Phe/Leu Ile/Leu/Val Pro/Leu Ala/Val. . . NO. 23. Additionally, the protoxin segments of the cryI toxins (which follow residue 601) bear more sequence similarity than the "toxin" segments. Because of this sequence similarity, the transition point in the protoxin segment for making a "chimeric" protein between the cryIF sequence and the crylA(b) or 436 sequence can be readily determined by one skilled in the. . .

DETD(51) Therefore a "chimeric" "toxin" of the subject invention can comprise the full cryIF "toxin" and a portion of the cryIF protoxin, transitioning to the corresponding crylA(b) or 436 sequence at any position between the end of the "toxin" segment (as defined above) and the end of the peptide sequence shown in SEQ ID NO. 31. Preferably, the amino acid sequence of the C-terminus of the "chimeric" "toxin" comprises a crylA(b) sequence or a sequence from the 436 gene or an equivalent of one of these sequences.

DETD(53) FIG. . . . used in the toxins of the subject invention. SEQ ID NO. 35 shows the amino acid sequence of a CryIF/CrylA(b) "chimeric" "toxin" of the subject invention that corresponds to the "Cons" sequence shown in FIG. 9. SEQ ID NO. 36 shows the amino acid sequence of a "chimeric" "toxin" of the subject invention that incorporates the alternative amino acids as shown in the first "Alt" sequence listed above the "Cons" sequence shown in FIG. 9. SEQ ID NO. 37 shows the amino acid sequence of a "chimeric" "toxin" of the subject invention that incorporates the alternative amino acids as shown in the second "Alt" sequence listed above the first "Alt" sequence shown in FIG. 9. SEQ ID NO. 38 shows the amino acid sequence of a "chimeric" "toxin" of the subject invention that incorporates the alternative amino acids as shown in the third "Alt" sequence listed above the. . . shown in FIG. 9. It is also well known in the art that various mutations can be made in a "toxin" sequence without changing the activity of a "toxin". Furthermore, due to the degeneracy of the genetic code, a variety of DNA sequences can be used to encode a particular "toxin". These alternative DNA and amino acid sequences can be used according to the subject invention by a person skilled in. . .

DETD(55) The . . . can be carried out according to the subject invention. BamHI and PvuI cloning sites can be introduced into a crylA(c)/crylA(b) "chimeric" "toxin" gene by mutagenesis using the PCR technique of Splice Overlap Extension (SOE) (Horton, R. M., H. D. Hunt, S. N. . . pMYC2224. The new plasmid, which we designated pMYC2239, consisted of a short segment of crylA(c) followed by cryIF to the "toxin"/protoxin segment junction. Thus, the protoxin segment was now derived from crylA(b) (pMYC1050). An ApaI fragment derived from the cryIF clone. . . substituted for the ApaI fragment in pMYC2239. The resulting clone (pMYC2244) consisted of cryIF from the initiator methionine to the "toxin"/protoxin segment junction and crylA(b) to the end of the coding region. Clone pMYC2243 was constructed by SOE to introduce silent. . . from pMYC2243 that contained the silent changes was substituted for the ApaI fragment in pMYC2244 to give clone pMYC2523. The "chimeric" pMYC2523 showed an expression improvement over pMYC2243, which contains unchanged cryIF protein sequence.

DETD(68) Treatment of cells. Bacillus "thuringiensis" or recombinant cells expressing the B.t. toxins can be treated to prolong the "toxin" activity and stabilize the cell. The pesticide microcapsule that is formed comprises the B.t. "toxin" or toxins within a cellular structure that has been stabilized and will protect the "toxin" when the microcapsule is applied to the environment of the target pest. Suitable host cells may include either prokaryotes or. . .

DETD(116) A "toxin"-containing DNA fragment was generated by PCR with primers L/D on template pMYC1260. The DNA was digested with BglII and PvuI. . . correct plasmids were identified by PCR analysis and agarose-TBE gel electrophoresis using the primer set N/O, which bridges the BamHI/BglII "fusion" junction.

DETD(151) A second type of "chimeric" "toxin" was assembled by substituting the 436 protoxin module for the crylA(b) protoxin in pMYC2523 (FIG. 8). The 436 protoxin sequence. . .

DETD(159) Analysis for Synergy Between CryIF "Chimeric" "Toxin" and CrylA(c) "Chimeric" "Toxin" Against the Corn Earworm, Heliothis zea

DETD(170) TABLE 2 __

% INHIBITION					
cryIF/	crylA(c)/				
	1:1 mix of the two				
Rate	crylA(b)	crylA(b)	"chimeric" toxins		
.mu.g "toxin"/g diet					
a	b	E(exp)	E(obs)	SF	
50.0	--	--	50	78	1.6
25.0	13	23	22	62	2.8. . .

DETD(172) Analysis for Synergy Between CryIF "Chimeric" "Toxin" and CrylA(c) "Chimeric" "Toxin" Against the Corn Earworm, Heliothis zea

We claim:
1. A composition for controlling lepidopteran pests, wherein said composition comprises cells which express a CryIF "chimeric" core "toxin"-containing protein and a CrylA(c) "chimeric" core "toxin"-containing protein.

2. The composition, according to claim 1, comprising a cell expressing a CryIF "chimeric" core "toxin"-containing protein and a cell expressing a CrylA(c) "chimeric" core "toxin"-containing protein.

3. The composition, according to claim 1, comprising a cell expressing a CryIF "chimeric" core "toxin"-containing protein and a CrylA(c) "chimeric" core "toxin"-containing protein.

4. The composition, according to claim 1, wherein said CryIF "chimeric" core "toxin"-containing protein comprises a CryIF core N-terminal protein portion and a heterologous C-terminal "toxin" portion from a CrylA(b) "toxin" or CrylA(b)/CrylA(c) "chimeric" "toxin".

5. The composition, according to claim 4, wherein said CryIF "chimeric" core "toxin"-containing protein has approximately 1150 to 1200 amino acids and comprises a CryIF core N-terminal sequence of at least about 590. . .

10. The composition, according to claim 6, wherein said CryIF "chimeric" core "toxin"-containing protein comprises an amino acid sequence selected from the group consisting of SEQ ID NO. 35, SEQ ID NO. 36,. . .

11. The composition, according to claim 1, wherein said CrylA(c) "chimeric" core "toxin"-containing protein has an amino acid sequence shown in SEQ ID NO. 34.

14. A host transformed to express both a CryIF "chimeric" core "toxin"-containing protein and a CrylA(c) "chimeric" core "toxin"-containing protein, wherein said host is a microorganism or a plant cell.

15. . . . pests, or the environment of said pests, with an effective amount of a composition comprising cells which produce a CryIF "chimeric" core "toxin"-containing protein and a CrylA(c) "chimeric" core "toxin"-containing protein.

16. The method, according to claim 15, wherein said composition comprises a cell expressing a CryIF "chimeric" core "toxin"-containing protein and a cell expressing a CrylA(c) "chimeric" core "toxin"-containing protein.

17. The method, according to claim 15, wherein said composition comprises a cell expressing a CryIF "chimeric" core "toxin"-containing protein and a CrylA(c) "chimeric" core "toxin"-containing protein.

18. The method, according to claim 15, wherein said CryIF "chimeric" core "toxin"-containing protein comprises a CryIF core N-terminal "toxin" portion and a heterologous C-terminal protoxin portion from a CrylA(b) "toxin" or CrylA(b)/CrylA(c) "chimeric" "toxin".

19. The method, according to claim 18, wherein said CryIF "chimeric" core "toxin"-containing protein has approximately 1150 to 1200 amino acids and comprises a CryIF core N-terminal sequence of at least about 590. . .

24. The method, according to claim 20, wherein said CryIF "chimeric" core "toxin"-containing protein comprises an amino acid sequence selected from the group consisting of SEQ ID NO. 35, SEQ ID NO. 36,. .

25. The method, according to claim 18, wherein said CrylA(c) "chimeric" core "toxin"-containing protein has an amino acid sequence shown in SEQ ID NO. 34.

US PAT NO: 5,055,294 [IMAGE AVAILABLE] L6: 122 of 128

I claim:
1. DNA encoding a B.t. toxin having the amino acid sequence shown in FIG. 2.

2. DNA, according to claim 1, having the nucleotide sequence shown in FIG. 1.

3. DNA, according to claim 1, having the nucleotide sequence shown in FIG. 1, wherein said sequence terminates at the stop codon.

4. A recombinant DNA transfer vector comprising DNA which codes for the amino acid sequence shown in FIG. 2.

5. The DNA transfer vector, according to claim 4, transferred to and replicated in a prokaryotic or eukaryotic host.

6. A bacterial host transformed to express a B.t. toxin having the amino acid sequence shown in FIG. 2.

7. Pseudomonas fluorescens, according to claim 6, transformed with a plasmid vector containing the B.t. toxin gene encoding the B.t. toxin having the amino acid sequence shown in FIG. 2.

8. Pseudomonas fluorescens (pM3,130-7), having the identifying characteristic of NRRL B-18332, a Pseudomonas fluorescens according to claim 7.

9. A microorganism according to claim 6, which is a species of Pseudomonas, Azotobacter, Erwinia, Serratia, Klebsiella, Rhizobium, Rhodopseudomonas, Methylophilius, Agrobacterium, Acetobacter or Alcaligenes.

10. A microorganism according to claim 9, wherein said microorganism is pigmented and phylloplane adherent.

11. A method for controlling lepidopteran insects which comprises administering to said insects or to the environment of said insects a microorganism according to claim 9.

12. A method according to claim 11, wherein said administration is to the rhizosphere.

13. A method according to claim 12, wherein said administration is to the phylloplane.

14. A method according to claim 11, wherein said administration is to a body of water.

15. An insecticidal composition comprising insecticide containing substantially intact, treated cells having prolonged pesticidal activity when applied to the environment of a target pest, wherein said insecticide is a polypeptide toxic to lepidopteran insects, is intracellular, and is produced as a result of expression of a transformed microbe capable of expressing the B.t. toxin having the amino acid sequence shown in FIG. 2.

16. The insecticidal composition, according to claim 15, wherein said treated cells are treated by chemical or physical means to prolong the insecticidal activity in the environment.

17. The insecticidal composition, according to claim 16, wherein said cells are prokaryotes or lower eukaryotes.

18. The insecticidal composition, according to claim 17, wherein said prokaryote cells are selected from the group consisting of Enterobacteriaceae, Bacillaceae, Rhizobiaceae, Spirillaceae, Lactobacillaceae, Pseudomonadaceae, Azotobacteraceae, and Nitrobacteraceae.

19. The insecticidal composition, according to claim 17, wherein said lower eukaryote cells are selected from the group consisting of Phycomycetes, Ascomycetes, and Basidiomycetes.

20. The insecticidal composition, according to claim 15, wherein said cell is a pigmented bacterium, yeast, or fungus.

21. Treated, substantially intact unicellular microorganism cells containing an intracellular "toxin", which "toxin" is a result of expression of a Bacillus "thuringiensis" "toxin" gene toxic to lepidopteran insects which codes for a polypeptide "toxin" having the amino acid sequence shown in FIG. 2, wherein said cells are treated under conditions which prolong the insecticidal activity when said cell is applied to the environment of a target ins

22. The cells, according to claim 21, wherein the cells are treated by chemical or physical means to prolong the insecticidal activity in the environment.

23. The cells according to claim 21, wherein said microorganism is Pseudomonas and said toxin is a B.t. toxin having the amino acid sequence shown in FIG. 2.

24. Pseudomonas cells according to claim 23, wherein said cells are treated with iodine.

25. The cells, according to claim 21, which are Pseudomonas fluorescens.

26. The cells, according to claim 25, which are Pseudomonas fluorescens (pM3,130-7).

27. A plasmid selected from the group consisting of pM2,107-1, pM3,123-1 and pM3,130-7.

28. Plasmid pM3,130-7, according to claim 27.

US PAT NO: 5,010,001 [IMAGE AVAILABLE] L6: 124 of 128

What is claimed is:
1. A method for producing B. "thuringiensis" delta-"endotoxin" in enhanced amounts which comprises: growing transformed E. coli in an appropriate nutrient medium, wherein said E. coli are transformed with an expression vector containing the intact structural gene encoding said delta-"endotoxin" and 3' and 5' flanking regions which do not extend beyond the proximal HincII sites; and isolating the expressed delta-"endotoxin".

2. A method according to claim 1, wherein said 3'-flanking region is less than about 300 bp, and the 5'-flanking region extends to the 5'-upstream HincII site.

3. A method according to claim 2, wherein said 3'-flanking region is at least partially removed with an exonuclease from said HincII cleavage site, and the 5'-flanking region extends to the 5'-upstream HincII site.

4. A DNA fragment containing the intact structural gene encoding the delta-*endotoxin* of B. *thuringiensis* and 3' and 5' flanking regions which do not extend beyond the proximal HincII sites.

5. A DNA construct comprising an E. coli replicon and a DNA fragment according to claim 4.

6. A DNA construct according to claim 5, wherein said replicon is derived from pBR322.

7. E. coli transformed with a construct according to claim 5.

US PAT NO: H 875 [IMAGE AVAILABLE] L6: 126 of 128
What is claimed is:
1. A nucleic acid fragment comprising a nucleic acid sequence encoding a soluble insecticidal protein, wherein at least one of the positively charged amino acids selected from the group consisting of lysine, arginine, aspartate and glutamate is instead a negatively charged or neutral amino acid.

2. A nucleic acid fragment according to claim 1 wherein at least one of the positively charged amino acids is instead a neutral amino acid.

3. A nucleic acid fragment according to claim 2 wherein the neutral amino acid is alanine.

4. A nucleic acid fragment according to claim 3 wherein arginine 25 is instead alanine 25, arginine 30 is instead alanine 30, arginine 78 is instead alanine 78, or lysine 124 is instead alanine 124.

5. A nucleic acid fragment according to claim 4 wherein arginine 25 is instead alanine 25.

6. A nucleic acid fragment according to claim 4 wherein arginine 30 is instead alanine 30.

7. A nucleic acid fragment according to claim 4 wherein arginine 78 is instead alanine 78.

8. A nucleic acid fragment according to claim 4 wherein lysine 124 is instead alanine 124.

9. A nucleic acid fragment according to claim 1 which is a DNA fragment.

10. A nucleic acid fragment according to claim 1 wherein the molecular weight of the encoded solubilized insecticidal protein is about 27 kDa.

11. A microorganism selected from the group consisting of Bacillus megaterium, Bacillus subtilis and Bacillus thuringiensis containing a nucleic acid fragment according to claim 1.

L10 1. 5,908,970, Jun. 1, 1999, Recombinant plant expressing non-competitively binding Bt insecticidal crystal proteins; Herman Van Mellaert, et al., 435/320.1, 419; 536/23.71 [IMAGE AVAILABLE] APPL-NO: 08/463,240 DATE FILED: Jun. 5, 1995 FRN-PR. NO: 89401499 FRN FILED: May 31, 1989 FRN-PR. CO: United Kingdom REL-US-DATA: Division of Ser. No. 173,274, Dec. 23, 1993, abandoned, which is a continuation of Ser. No. 640,400.

2. 5,859,328, Jan. 12, 1999, Isolated DNA elements that direct pistil-specific and anther-specific gene expression and methods of using same; Mikhail E. Nasrallah, et al., 800/287; 435/69.1, 70.1, 320.1; 536/24.1; 800/286, 294, 298, 303 [IMAGE AVAILABLE] APPL-NO: 08/485,158 DATE FILED: Jun. 7, 1995 REL-US-DATA: Continuation of Ser. No. 54,362, May 3, 1993, abandoned.

3. 5,843,898, Dec. 1, 1998, Transformation vectors allowing expression of foreign polypeptide endotoxins in plants; Henri Marcel Jozef De Greve, et al., 514/12; 435/69.1 [IMAGE AVAILABLE] APPL-NO: 08/463,510 DATE FILED: Jun. 5, 1995 REL-US-DATA: Division of Ser. No. 446,486, May 22, 1995, Pat. No. 5,545,565, which is a continuation of Ser. No. 133,965, Oct. 8, 1993, abandoned, which is a division of Ser. No. 14,148, Feb. 5, 1993, Pat. No. 5,317,096, which is a division of Ser. No. 555,828, Jul. 23, 1990, Pat. No. 5,254,799, which is a continuation of Ser. No. 821,582, Jan. 22, 1986, abandoned, which is a continuation-in-part of Ser. No. 692,759, Jan. 18, 1985,abandoned.

4. 5,840,554, Nov. 24, 1998, .beta.-Endotoxin expression in pseudomonas fluorescens; Mark Thompson, et al., 435/471; 424/405, 538; 435/69.7, 252.34, 320.1, 480; 514/2; 530/350; 536/23.4, 23.71 [IMAGE AVAILABLE] APPL-NO: 08/639,923 DATE FILED: Apr. 24, 1996 REL-US-DATA: Division of Ser. No. 239,476, May 6, 1994, Pat. No. 5,527,883.

5. 5,827,514, Oct. 27, 1998, Pesticidal compositions; Gregory A. Bradfisch, et al., 424/93.2, 93.1, 93.3; 435/69.1, 69.7, 252.3, 410, 418, 419 [IMAGE AVAILABLE] APPL-NO: 08/598,305 DATE FILED: Feb. 8, 1996 REL-US-DATA: Continuation of Ser. No. 349,867, Dec. 6, 1994, Pat. No. 5,508,264.

6. 5,824,302, Oct. 20, 1998, Method of controlling insect larvae comprising feeding an insecticidal amount of a transgenic maize plant expressing a polypeptide having Bt-crystal protein toxic properties; Gleta Carswell, et al., 424/93.21, 93.2, 93.461; 536/23.71; 800/302 [IMAGE AVAILABLE] APPL-NO: 8/768,325 DATE FILED: Dec. 17, 1996 REL-US-DATA: Continuation of Ser. No. 445,526, May 22, 1995, Pat. No. 5,595,733, which is a division of Ser. No. 269,677, Jul. 1, 1994, which is a continuation of Ser. No. 24,875, Mar. 1, 1993, Pat. No. 5,350,689, Sep. 27, 1994, which is a continuation of Ser. No. 276,210, Nov. 23, 1988, abandoned, which is a continuation-in-part of Ser. No. 178,170, Apr. 6, 1988, abandoned, which is a continuation-in-part of Ser. No. 56,552, May 29, 1987, abandoned, and Ser. No. 56,506, May 29, 1987, abandoned, which is a continuation-in-part of Ser. No. 53,241, May 22, 1987, abandoned, which is a continuation-in-part of Ser. No. 52,440, May 20, 1987, abandoned.

7. 5,770,450, Jun. 23, 1998, Zea mays plants regenerated from protoplasts or protoplast-derived cells; Ray Shillito, et al., 435/424, 412, 421, 430.1, 431 [IMAGE AVAILABLE] APPL-NO: 08/269,677 DATE FILED: Jul. 1, 1994 REL-US-DATA: Continuation of Ser. No. 24,875, Mar. 1, 1993, Pat. No. 5,350,689, which is a continuation of Ser. No. 276,210, Nov. 23, 1988, abandoned, which is a continuation-in-part of Ser. No. 178,170, Apr. 6, 1988, abandoned, which is a continuation-in-part of Ser. No. 56,552, May 29, 1987, abandoned, and Ser. No. 56,506, May 29, 1987, abandoned, which is a continuation-in-part of Ser. No. 53,241, May 22, 1987, abandoned, which is a continuation-in-part of Ser. No. 52,440, May 20, 1987, abandoned.

8. 5,767,372, Jun. 16, 1998, Transformation vectors allowing expression of foreign polypeptide endotoxins from Bacillus *thuringiensis* in plants; Henri Marcel Jozef De Greve, et al., 800/302; 435/320.1, 419; 536/23.71 [IMAGE AVAILABLE] APPL-NO: 08/463,308 DATE FILED:Jun. 5, 1995 REL-US-DATA: Division of Ser. No. 446,486, May 22, 1995, Pat. No. 5,545,565, which is a continuation of Ser. No. 133,965, Oct. 8, 1993, abandoned, which is a division of Ser. No. 14,148, Feb. 5, 1993, Pat. No. 5,317,096, which is a division of Ser. No. 555,828, Jul. 23, 1990, Pat. No. 5,254,799, which is a continuation of Ser. No. 821,582, Jan. 22, 1986, abandoned, which is a continuation-in-part of Ser. No. 692,759, Jan. 18, 1985, abandoned.

9. 5,766,900, Jun. 16, 1998, Method of regenerating fertile transgenic Zea mays plants from protoplasts; Ray Shillito, et al., 800/292; 47/DIG.1 [IMAGE AVAILABLE] APPL-NO: 08/418,810 DATE FILED: Apr. 7, 1995 REL-US-DATA: Division of Ser. No. 269,677, Jul. 1, 1994, which is a continuation of Ser. No. 24,875, Mar. 1, 1993, Pat. No. 5,350,689, which is a continuation of Ser. No. 276,210, Nov. 23, 1988, abandoned, which is a continuation-in-part of Ser. No. 178,170, Apr. 6, 1988, abandoned, which is a continuation-in-part of Ser. No. 56,552, May 29, 1987, abandoned, and Ser. No. 56,506, May 29, 1987, abandoned, said Ser. No. 56,506 is a continuation-in-part of Ser. No. 53,241, May 22, 1987, abandoned, which is a continuation-in-part of Ser. No. 52,440, May 20, 1987, abandoned

10. 5,763,241, Jun. 9, 1998, Insect resistant plants; David A. Fischhoff, et al., 800/279; 435/418, 419; 536/23.71 [IMAGE AVAILABLE] APPL-NO: 08/759,446 DATE FILED: Dec. 5, 1996 REL-US-DATA: Continuation of Ser. No. 435,101, May 4, 1995, abandoned, which is a division of Ser. No. 72,281, Jun. 4, 1993, Pat. No. 5,495,071, which is a continuation of Ser. No. 523,284, May 14, 1990, abandoned, which is a continuation of Ser. No. 44,081, Apr. 29, 1987, abandoned

11. 5,760,181, Jun. 2, 1998, Endotoxins; Henri Marcel Jozef De Greve, et al., 530/350 [IMAGE AVAILABLE] APPL-NO: 08/744,532 DATE FILED: Nov. 6, 1996 REL-US-DATA: Division of Ser. No. 463,308, Jun. 2, 1995, which is a continuation of Ser. No. 133,965, Oct. 8, 1993, abandoned, which is a division of Ser. No. 14,148, Feb. 5, 1993, Pat. No. 5,317,096, which is a division of Ser. No. 555,828, Jul. 23, 1990, Pat. No. 5,254,799, which is a continuation of Ser. No. 821,582, Jan. 22, 1986, abandoned, which is a continuation-in-part of Ser. No. 692,759, Jan. 18, 1985, abandoned.

12. 5,723,756, Mar. 3, 1998, Bacillus *thuringiensis* strains and their genes encoding insecticidal toxins; Marnix Peferoen, et al., 800/279; 435/69.1, 410; 536/23.71; 800/294, 301, 317.2 [IMAGE AVAILABLE] APPL-NO: 08/443,679 DATE FILED: May 18, 1995 FRN-PR. NO: 90401144 FRN FILED Apr. 26, 1990 FRN-PR. CO: United Kingdom FRN-PR. NO: 90403724 FRN FILED: Dec. 20, 1990 FRN-PR. CO: United Kingdom REL-US-DATA: Division of Ser. No. 952,755, Nov. 17, 1992, Pat. No. 5,466,597.

13. 5,625,136, Apr. 29, 1997, Synthetic DNA sequence having enhanced insecticidal activity in maize; Michael G. Koziel, et al., 800/302; 435/69.1; 536/23.1, 23.71 [IMAGE AVAILABLE] APPL-NO: 07/951,715 DATE FILED: Sep. 25, 1992 REL-US-DATA: Continuation-in-part of Ser. No. 772,027, Oct. 4, 1991, abandoned

14. 5,614,395, Mar. 25, 1997, Chemically regulatable and anti-pathogenic DNA sequences and uses thereof; John A. Ryals, et al., 435/6, 4, 69.1, 468; 536/24.1; 800/279 [IMAGE AVAILABLE] APPL-NO: 08/181,271 DATE FILED: Jan. 13, 1[illegible] REL-US-DATA: Continuation-in-part of Ser. No. 93,301, Jul. 16, 1993, abandoned, Ser. No. 42,847, Apr. 6, 1993, abandoned, Ser. No. 45,957, Apr. 12, 1993, abandoned, and Ser. No. 848,506, Mar. 6, 1992, abandoned, which is a continuation-in-part of Ser. No. 768,122, Sep. 27, 1991, abandoned, which is a continuation-in-part of Ser. No. 580,431, Sep. 7, 1990, abandoned, which is a continuation-in-part of Ser. No. 425,504, Oct. 20, 1989, abandoned, which is a continuation-in-part of Ser. No. 368,672, Jun. 20, 1989, abandoned, which is a continuation-in-part of Ser. No. 329,018, Mar. 24, 1989, abandoned, said Ser. No. 93,301 is a continuation of Ser. No. 973,197, Nov. 6, 1992, abandoned, which is a continuation of Ser. No. 678,378, Apr. 1, 1991, abandoned, which is a continuation of Ser. No. 305,566, Feb. 6, 1989, abandoned, which is a continuation-in-part of Ser. No. 165,667, Mar. 8, 1988, abandoned, said Ser. No. 42,847 is a continuation of Ser. No. 632,441, Dec. 21, 1990, abandoned, which is a continuation-in-part of Ser. No. 425,504, Oct. 20, 1989, abandoned, and Ser. No. 165,667, Mar. 8, 1988, abandoned.

15. 5,593,881, Jan. 14, 1997, Bacillus *thuringiensis* delta-*endotoxin*; Mark Thompson, et al., 435/418, 252.3, 320.1; 536/23.71 [IMAGE AVAILABLE] APPL-NO: 08/239,474 DATE FILED: May 6, 1994

16. 5,545,565, Aug. 13, 1996, Transformation vectors allowing expression of fo[illegible] polypeptide endoxins from Bacillus *thuringiensis* in plants; Henri M. J. De Greve[illegible] al., 435/320.1, 69.1; 514/12; 536/23.71 [IMAGE AVAILABLE] APPL-NO: 08/446,486 DATE FILED: May 22, 1995 REL-US-DATA: Continuation of Ser. No. 133,965, Oct. 8, 1993, abandoned, which is a division of Ser. No. 14,148, Feb. 5, 1993, Pat. No. 5,317,096, which is a division of Ser. No. 555,828, Jul. 23, 1990, Pat. No. 5,254,799, which is a continuation of Ser. No. 821,582, Jan. 22, 1986, abandoned, which is a continuation-in-part of Ser. No. 692,759, Jan. 18, 1985, abandoned.

17. 5,527,883, Jun. 18, 1996, Delta-endotoxin expression in pseudomonas fluorescens; Mark Thompson, et al., 530/350; 435/252.34, 320.1; 536/23.4, 23.71 [IMAGE AVAILABLE] APPL-NO: 08/239,476 DATE FILED: May 6, 1994

18. 5,516,693, May 14, 1996, Hybrid gene incorporating a DNA fragment containing a gene coding for an insecticidal protein, plasmids, transformed cyanobacteria expressing such protein and method for use as a biocontrol agent; Mark A. Vaeck, et al., 435/320.1, 69.7, 252.33; 536/23.4, 23.71 [IMAGE AVAILABLE] APPL-NO: 07/021,405 DATE FILED: Mar. 4, 1987

19. 5,508,264, Apr. 16, 1996, Pesticidal compositions; Gregory A. Bradfisch, et al., 514/12; 530/350 [IMAGE AVAILABLE] APPL-NO: 08/349,867 DATE FILED: Dec. 6, 1994

20. 5,500,365, Mar. 19, 1996, Synthetic plant genes; David A. Fischhoff, et al., 435/418, 411, 414, 417; 536/23.71 [IMAGE AVAILABLE] APPL-NO: 07/959,506 DATE FILED: Oct. 9, 1992 REL-US-DATA: Continuation of Ser. No. 476,661, Feb. 12, 1990, abandoned, which is a continuation-in-part of Ser. No. 315,355, Feb. 24, 1989, abandoned.

21. 5,495,071, Feb. 27, 1996, Insect resistant tomato and potato plants; David A. Fischhoff, et al., 800/302; 435/69.1, 320.1, 411, 417, 418; 514/12; 536/23.71 [IMAGE AVAILABLE] APPL-NO 08/072,281 DATE FILED: Jun. 4, 1993 REL-US-DATA: Continuation of Ser. No. 523,284, May 14, 1990, abandoned, which is a continuation of Ser. No. 44,081, Apr. 29, 1987, abandoned.

22. 5,466,785, Nov. 14, 1995, Tissue-preferential promoters; Annick J. de Framond, 536/24.1; 424/93.2; 435/320.1; 536/23.7 [IMAGE AVAILABLE] APPL-NO: 08/322,962 DATE FILED: Oct. 13, 1994 REL-US-DATA: Continuation of Ser. No. 71,209, Jun. 2, 1993, abandoned, which is a continuation of Ser. No. 508,207, Apr. 12, 1990.

23. 5,350,689, Sep. 27, 1994, Zea mays plants and transgenic Zea mays plants regenerated from protoplasts or protoplast-derived cells; Ray Shillito, et al., 435/412, 421 [IMAGE AVAILABLE] APPL-NO: 08/024,875 DATE FILED: Mar. 1, 1993 REL-US-DATA: Continuation of Ser. No. 276,210, Nov. 23, 1988, abandoned, which is a continuation-in-part of Ser. No. 178,170, Apr. 6, 1988, abandoned, which is a continuation-in-part of Ser. No. 56,552, May 29, 1987, abandoned, and a continuation-in-part of Ser. No. 56,506, May 29, 1987, abandoned, which is a continuation-in-part of Ser. No. 53,241, May 22, 1987, abandoned, which is a continuation-in-part of Ser. No. 52,440, May 20, 1987, abandoned.

24. 5,317,096, May 31, 1994, Transformation vectors allowing expression of foreign polypeptide endotoxins from Bacillus *thuringiensis* in plants; Henri M. J. De Greve, et al., 536/23.71 [IMAGE AVAILABLE] APPL-NO: 08/014,148 DATE FILED: Feb. 5, 1993 REL-US-DATA: Division of Ser. No. 555,828, Jul. 23, 1990, which is a continuation of Ser. No. 821,582, Jan. 22, 1986, abandoned, which is a continuation-in-part of Ser. No. 692,759, Jan. 18, 1985, abandoned.

25. 5,306,628, Apr. 26, 1994, Method and means for extending the host range of insecticidal proteins; Natarajan Sivasubramanian, et al., 435/69.7, 320.1; 530/350; 536/23.71 [IMAGE AVAILABLE] APPL-NO: 07/829,902 DATE FILED: Feb. 3, 1992 REL-US-DATA: Division of Ser. No. 518,575, May 3, 1990, Pat. No. 5,143,905.

26. 5,254,799, Oct. 19, 1993, Transformation vectors allowing expression of Bacillus *thuringiensis* endotoxins in plants; Henri M. J. De Greve, et al., 800/302; 435/418 [IMAGE AVAILABLE] APPL-NO: 07/555,828 DATE FILED: Jul. 23, 1990 REL-US-DATA: Continuation of Ser. No. 821,582, Jan. 22, 1986, abandoned, which is a continuation-in-part of Ser. No. 692,759, Jan. 18, 1985, abandoned.

27. 5,177,308, Jan. 5, 1993, Insecticidal toxins in plants; Kenneth A. Barton, et al., 800/302; 435/320.1 [IMAGE AVAILABLE] APPL-NO: 07/443,425 DATE FILED: Nov. 29, 1989 APPL-NO: 07/164,162 DATE FILED: Mar. 3, 1988

28. 5,143,905, Sep. 1, 1992, Method and means for extending the host range of insecticidal proteins; Natarajan Sivasubramanian, et al., 514/21; 424/405; 435/69.7; 514/8, 12; 530/350, 409 [IMAGE AVAILABLE] APPL-NO: 07/518,575 DATE FILED: May 3, 1990

29. 5,055,294, Oct. 8, 1991, *Chimeric* Bacillus *thuringiensis* crystal protein gene comprising HD-73 and Berliner 1715 *toxin* genes, transformed and expressed in Pseudomonas fluorescens; Thomas E. Gilroy, 424/93.2, 93.21; 435/69.1, 69.7, 252.3, 252.31, 252.32, 252.33, 252.34, 254.11, 254.2, 320.1; 536/23.71 [IMAGE AVAILABLE]
APPL-NO: 07/164,162 DATE FILED: Mar. 3, 1988

30. H 875, Jan. 1, 1991, *Toxin*-encoding nucleic acid fragments derived from a Bacillus *thuringiensis* subsp. israelensis gene; David J. Ellar, et al., 435/252.31, 69.1, 252.5, 832; 530/350, 858; 536/23.7, 23.71 [IMAGE AVAILABLE] APPL-NO: 07/170,211 DATE FILED: Mar. 18, 1988

31. 4,945,057, Jul. 31, 1990, Monoclonal antibodies to crystal protein of Bacillus *thuringiensis* subspecies israelensis; Kevin B. Temeyer, et al., 530/388.4; 435/70.21, 340, 832, 948; 436/548; 530/809, 825 [IMAGE AVAILABLE] APPL-NO: 07/050,451 DATE FILED: May 18, 1987

US PAT NO: 5,908,970 [IMAGE AVAILABLE] L10: 1 of 31
ABSTRACT: Plants . . . to insects by transforming their nuclear genome with two or more DNA sequences, each encoding a different non-competitively binding B. *thuringiensis* protoxin or insecticidal part thereof, preferably the *toxin* thereof.

BSUM(8) For example, Goldman et al. (1986) have applied selection with B. *thuringiensis* israelensis *toxin* over 14 generations of Aedes aegypti and found only a marginal decrease in sensitivity. The lack of any observable trend. . . *Mosquito Control Research, Annual Report 1983, University of California.") with Culex quinquefasciatus obtained an 11-fold increase in resistance to B. *thuringiensis* israelensis after 32 generations at LC.sub.95 selection presssure.

BSUM(15) Most of the anti-Lepidopteran B. *thuringiensis* (e.g., Bt3, Bt2, Bt73, Bt14, Bt15, Bt4, Bt18) ICP genes encode 130 to 140 kDa protoxins which dissolve in the alkaline environment of an insect's midgut and are proteolytically activated into an active *toxin* of 60-65 kDa. These ICPs are related and can be recognized as members of the same family based on sequence. . .

BSUM(17) It has recently become clear that heterogeneity exists also in the anti-Coleopteran *toxin* gene family. Whereas several previously reported *toxin* gene sequences from different B. *thuringiensis* isolates with anti-Coleopteran activity were identical (EP 0149162 and 0202739), the sequences and structure of bt21 and bt22 are substantially.

BSUM(18) While the insecticidal spectra of B. *thuringiensis* ICPs are different, the major pathway of their toxic action is believed to be common. All B. *thuringiensis* ICPs, for which the mechanism of action has been studied in any detail, interact with the midgut epithelium of sensitive. . . brush border membrane and the osmotic balance over this membrane are perturbed. In the pathway of toxic action of B. *thuringiensis* ICPs, the binding of the *toxin* to receptor sites on the brush border membrane of these cells is an important feature (Hofmann et al., 1988b). The *toxin* binding sites in the midgut can be regarded as an ICP-receptor since *toxin* is bound in a saturable way and with high affinity (Hofmann et al., 1988a).

DETD(3) As used herein, "B. *thuringiensis* ICP" (or "ICP") should be understood as an intact protein or a part thereof which has insecticidal activity and which can be produced in nature by B. *thuringiensis*. An ICP can be a protoxin, as well as an active *toxin* or another insecticidal truncated part of a protoxin which need not be crystalline and which need not be a naturally occurring protein. In this regard, an ICP can be a chimaeric *toxin* encoded by the combination of two variable regions of two different ICP genes as disclosed in EP 0228838.

DETD(7) As used herein, "truncated B. *thuringiensis* gene" should be understood as a fragment of a full-length B. *thuringiensis* gene which still encodes at least the toxic part of the B. *thuringiensis* ICP, preferentially the *toxin*.

DETD(11) A "receptor" should be understood as a molecule, to which a ligand (here a B. *thuringiensis* ICP, preferably a *toxin*) can bind with high affinity (typically a dissociation constant (Kd) between 10.sup.-11 and 10.sup.-6 M) and saturability. A determination of. . .

DETD(19) To . . . 1983), and provided with suitable translation initiation sites (e.g., Stanssens et al, 1985 and 1987). Gene cassettes of the B. *thuringiensis* ICP genes can be generated by site-directed mutagenesis, for example according to the procedure described by Stanssens et al. (1985 and. . . 1987). This allows cassettes to be made comprising, for example, a truncated ICP gene fragment encoding the toxic core (i.e., *toxin*) of an ICP or a hybrid gene encoding the toxic core and a selectable marker according to the procedures described.

DETD(45) In a first case, hybrid genes in which the coding region of one gene is in frame *fused* with the coding region of another gene can be placed under the control of a single promoter. *Fusions* can be made between either an ICP and a marker gene or between two ICP genes. An example of an ICP gene-marker gene *fusion* has been described in EP 0193259 (i.e., a hybrid truncated bt2-neo gene encoding a Bt2 *toxin*-NPTII *fusion* protein).

DETD(65) gene: A genomic library was prepared from total DNA of strain B. *thuringiensis* aizawai HD-68. Using the 1.1 kb internal HindIII fragment of the bt2 gene as a probe, a gene designated bt4. . . gene revealed an open reading frame of 3495 bp which encodes a protoxin of 132 kDa and a trypsin activated *toxin* fragment of 60 kDa. This (insect controlling protein) gene differs from previously identified genes and was also found in several. . .

DETD(68) The . . . gene has an open reading frame of 3567 bp which encodes a protoxin of 135 kDa and a 63 kDa *toxin* fragment. A similar gene has been described by Honee et al. 1988, isolated from B. *thuringiensis* entomocidus 60.5. The bt15 gene differs from the published sequence at three positions: an Ala codon (GCA) is present instead. . . positions are according to Honnee et al., 1988). Another similar gene has been described in EP 0295156, isolated from B. *thuringiensis* aizawai 7-29 and entomocidus 6-01. The bt15 gene is different from this published nucleotide sequence at three different places: 1). . .

DETD(69) The . . . It has an open reading frame of 3621 bp which encodes a 137 kDa protoxin and a 66 kDa activated *toxin* fragment. A similar gene has been cloned from B. *thuringiensis* HD-2 (Brizzard and Whiteley, 1988). The bt14 gene differs from the published nucleotide sequence by two nucleotide substitutions: a T. . .

DETD(109) By way of example for the Bt73 *toxin*, it has been shown that B. *thuringiensis* var. kurstaki HD73 produces a protein of 133 kDa encoded by a 6.6 kb type gene. A culture of this. . . collected and stored at -20.degree. C. until further use. Activation was performed according to Hofte et al. (1986). The purified *toxin* is further referred to as the Bt73 *toxin*.

DETD(216) Plasmid pGSH163, described in EP 0193259, contains the following *chimeric* genes between the T-DNA border repeats: a gene fragment encoding the *toxin* part of the bt2 gene under the control of the TR2' promoter and the neo gene under control of the. . .

DETD(217) A *chimeric* bt15 gene containing a gene fragment encoding the *toxin* of the Bt15 ICP under the control of the TR2' promoter, was constructed in the following way (FIG. 15). pOH50. . . a translational stop codon, was obtained by isolation of BclI-ClaI from pOH50 and cloning in pLK91, yielding pHW38. The whole *toxin* gene fragment was reconstructed under the control of the tac promoter, yielding pVE35, by ligation of a ClaI-PstI fragment from. . . pGSJ141 has been described in EPA 88402115.5. Ligation of the filled ClaI site to the filled NcoI site yielded a *chimeric* TR2'-truncated bt15 -3'g7 construct (pTVE47). As a selectable marker in this plasmid, the bar gene encoding phosphinothricin acetyl transferase and conferring resistance to PPT was used. A *chimeric* bar gene containing the bar gene under the control of the 35S promoter and followed by the end of. . .

CLMS(1) We . . . plant, comprising stably inserted into the genome of its cells, two to four DNA sequences each encoding a different Bacillus *thuringiensis* (Bt) insecticidal crystal protein (ICP) or an insecticidal portion thereof, toxic to the same insect species, wherein the encoded two. . .

CLMS(13) 13. The plant of claim 11, wherein said marker gene is *fused* with at least one of said two to four DNA sequences and is within the same transcriptional unit and under. . .

CLMS(20) 20. A plant cell, comprising stably inserted into its genome, two to four DNA sequences each encoding a different Bacillus *thuringiensis* (Bt) insecticidal crystal protein (ICP) or an insecticidal portion thereof, toxic to the same insect species, wherein the encoded two. . .

US PAT NO: 5,859,328 [IMAGE AVAILABLE] L10: 2 of 31 BSUM(6) Evidence for this specificity of SLG promoter activity derives from genetic ablation studies in which a *chimeric* gene construct consisting of the SLG promoter *fused* to the diphtheria *toxin* subunit A (DTA) gene was introduced into tobacco (M. K. Thorsness, M. K. Kandasamy, M. E. Nasrallah and J. B.. . . Nasrallah and J. B. Nasrallah, Plant Cell, Vol. 5, (1993) (in press)). Transformation of these plants with the SLG-DTA gene *fusion* resulted in the production at high frequency of transgenic plants that underwent normal differentiation and produced flowers in which only. . .

DETD(27) f) CytA *toxin* gene from Bacillus *thuringiensis* Israeliensis which encodes a protein that is mosquitocidal and hemolytic. When expressed in plant cells, it causes death of the. . .

CLMS(2) 2. A *chimeric* gene comprising a DNA element comprising SEQ ID NO:1, SEQ ID NO:2 and SEQ ID NO:3 operably linked to a. . .

CLMS(3) 3. A transgenic plant having integrated into its genome the *chimeric* gene of claim 2.

CLMS(5) 5. . . . comprises a gene selected from the group consisting of the pectate lyase gene pelE from Erwinia chrysanthemi EC16, the Diphtheria *toxin* A-chain gene, the T-urf 13 gene from cms-T maize mitochondrial genomes, the gin recombinase gene from phage Mu gene, the indole acetic acid-lysine synthetase gene from Pseudomonus syringae, and the CytA *toxin* gene from Bacillus *thuringiensis* Israeliensis.

CLMS(12) 12. A *chimeric* gene comprising a DNA element of the SLG13 promoter from the -339 to -79 region or from -339 to -143. . .

CLMS(15) 15. A transgenic plant having integrated into its genome the *chimeric* gene of claim 12.

CLMS(17) 17. . . . comprises a gene selected from the group consisting of the pectate lyase gene PelE from Erwinia chrysanthemi EC16, the Diphtheria *toxin* A-chain gene, the T-urf 13 gene from cms-T maize mitochondrial genomes, the gin recombinase gene from phage Mu gen, the indole acetic acid-lysine synthetase gene from Psuedomonus syringae, and the CytA *toxin* gene from Bacillus *thuringiensis* Israeliensis.

CLMS(21) 21. . . . polypeptide or RNA in a plant pistil, said method comprising growing a transgenic plant having integrated into its genome the *chimeric* gene claims 12.

US PAT NO: 5,843,898 [IMAGE AVAILABLE] L10: 3 of 31
ABSTRACT: Novel transformation vectors containing novel *chimeric* genes allow the introduction of exogenous DNA fragments coding for polypeptide toxins produced by Bacillus *thuringiensis* or having substantial sequence homology to a gene coding for a polypeptide *toxin* as described herein and expression of the *chimeric* gene in plant cells and their progeny after integration into the plant cell genome. Transformed plant cells and their progeny exhibit stably inherited polypeptide *toxin* expression useful for protecting said plant cells and their progeny against certain insect pests and in controlling said insect pests.

BSUM(2) This . . . the use of genetic engineering techniques in the modification of plants. More particularly, it concerns introduction and integration of a "chimeric" gene coding for a polypeptide "toxin" produced by Bacillus "thuringiensis" or having substantial sequence homology to a "toxin" gene described below in plant cells and obtaining an insect controlling level of expression of said polypeptide "toxin" intra-cellularly by transformed plant cells and their progeny.

BSUM(7) Bacillus "thuringiensis" (referred to at times herein as B.t.) bacteria includes approximately 19 known varieties that produce polypeptide toxins which form parasporal. . . by insect larvae, the crystals are solubilized and processed in the insect midgut to yield at least one active polypeptide "toxin" which is believed to act on the midgut cell membrane. Studies have revealed that individual crystal polypeptides exhibit insecticidal activity. . . .

BSUM(12) It is one object of this invention to provide novel "chimeric" genes coding for the polypeptide "toxin" produced by Bacillus "thuringiensis", or coding for a polypeptide "toxin" having substantial sequence homology to a "toxin" gene described herein. The "chimeric" genes' plant regulatory sequences direct expression in transformed plant cells.

BSUM(19) (b) at least one DNA fragment coding for a polypeptide "toxin" produced by Bacillus "thuringiensis" or having substantial sequence homology thereto.

BSUM(26) (ii) at least one DNA fragment coding for a polypeptide "toxin" produced by Bacillus "thuringiensis" or at least one DNA fragment having substantial sequence homology thereto.

BSUM(30) (b) at least one DNA fragment coding for a polypeptide "toxin" produced by Bacillus "thuringiensis", or at least one DNA fragment having substantial sequence homology thereto.

BSUM(34) Transformed plant cells and their progeny intra-cellularly express a polypeptide "toxin" substantially similar to the polypeptide toxins produced by Bacillus "thuringiensis" and are substantially toxic to certain insects. Transformed plant cells and their progeny may be used in controlling said insects.

DETD(7) (1) isolation of at least one DNA fragment from Bacillus "thuringiensis" coding for a polypeptide "toxin" by digestion of bacterial DNA and inserting the mixture of DNA fragments obtained into a cloning vehicle harbored in a. . .

DETD(25) Transformed plant cells and their progeny should express a polypeptide "toxin" substantially similar to polypeptide toxins being produced by Bacillus "thuringiensis" or a DNA fragment having substantial sequence homology to Bt2.

DETD(68) Straight promotor-gene "fusions" in which only part of the Bt2 coding sequence is used ("truncated Bt2"). Fragments of the Bt2 sequence still encoding an active "toxin" are inserted behind the plant specific promoters: The toxic polypeptides produced in the plant cells using these constructs should have. . .

DETD(71) Straight promotor-gene "fusions" in which a Bt:NPTII "fusion" gene (also referred to at times at Bt2:NPTII) is inserted behind the promotor. "Fusion" genes were constructed, consisting of a fragment of the Bt2 coding sequence (still encoding an active "toxin") "fused" to the coding sequence of the NPTII enzyme. The Bt:NPTII "fusion" genes used here, specify stable "fusion" proteins comprising amino terminal parts of the Bt2 protein "fused" to an intact Neomycin phosphotransferase (NTPII) enzyme. These "fusion" proteins have a specific toxicity comparable to the intact Bt2 protein and retain neomycin phosphotransferase enzyme activity. Thus, expression of the Bt:NPTII "fusion" proteins in plant cells allows direct selection for the production of this protein by isolating Kanamycin resistant (Km.sup.R) transformed cells.. . . to a high level of Kanamycin should identify, among all possible transformations, those which produce high levels of the toxic "fusion" protein. Further, expression of the "fusion" protein by a Bt:NPTII "fusion" gene might have other desirable properties such as stability in plant cells; for example, mRNA may be more stable. Differences in results obtained with these Type IV "fusion" genes might be due to intrinsic differences in the properties of the "fusion" protein expressed as compared to the intact Bt2 protein.

DETD(88) Kronstad et al., J. Bacteriol., 54, p. 419-428 (1983) reported that B.t. berliner 1715 contains two related "toxin" genes which are both located on plasmids. Intact "endotoxin" genes were isolated from a gene bank from total B.t. berliner 1715 plasmid DNA using partial Sau3A digests of plasmid. . . DNA. The pEcoR251 plasmid is a derivative of plasmid pBR322 in which the EcoRI-PuvII fragment has been replaced by a "chimeric" EcoRI endonuclease gene which is "fused" to a P.sub.R promotor fragment derived from plasmid pLK5 (Zabeau and Stanley, EMBO Journal, 1, 1217-1224 (1982)) as depicted in. . .

DETD(135) The previous data suggests that the smallest gene fragment of Bt2, encoding an active "toxin" is contained within the KpnI deletion fragment but extends further than the HindIII site. To map the exact endpoint of the minimal fragment coding for the active "toxin", deletion mutants were constructed which contained N-terminal fragments of decreasing size. To achieve this, we used a strategy which allowed us to construct simultaneously deletion-mutants and translational "fusions" to the NPTII-gene (see Section 7.2.2). The construction of the intermediate plasmid pLBKm25 is outlined in FIG. 18. As shown. . .

DETD(136) As . . . Bal31, cut with SalI, treated with Klenow polymerase and religated (FIG. 19). In this way, the deleted coding region is "fused" to a stopcodon with a minimum of nonsense coding sequence. An overview of the deletion clones is given in FIG.. . . blotting and ELISA for the quantitative detection of Bt2-like polypeptides and in an insect toxicity assay to screen for active "toxin". The results are presented in FIG. 21 and indicate that detection of a stable polypeptide decreases gradually when the endpoint. . .

DETD(141) Since NPTII is a most suitable selection marker in plant engineering, such gene "fusions" could have very promising applications. Indeed when using such NPTII "fusion" proteins to transform plants, a selection for high kanamycin resistance would allow direct selection for a high expression of the "fusion" product. Therefore, "toxin" gene "fusions" with NPTII might be used to transform plants and select for transformed plants expressing high levels of "toxin", by selection for kanamycin resistance.

DETD(170) Previous . . . on the Identification of minimal active toxic fragments have shown that this Kpn fragment comprises a (approximately 60 Kd) active "toxin" which exhibits the complete toxic activity of the Bt2 molecule. In the following, we wanted to determine whether the Bt:NPT2 "fusion" protein had still the same degree of toxicity.

DETD(176) 145 . . . concentrations. 8 transformants proved more resistant and were able to grow on concentrations higher than 200 ug/ml of kanamycin. The "fusion" point in all 8 clones was determined by restriction enzyme mapping with an accuracy of 20 bp. Surprisingly 7 out of 8 clones had their "fusion" point around the HindIII site at position 1680 of the Bt gene. One clone (pLBKm860) mapped at position approximately 2050. Although the majority of the deletions were "fused" around position 1800, none of these conferred a higher kanamycin resistant phenotype. The 7 clones which have their "fusion" point positioned around the HindIII site are too short to encode an active "toxin". However, one of the clones (pLBKm860) was:

DETD(186) Table . . . is the result of a cointegration of a receptor Ti plasmid with an intermediate vector. Each intermediate vector contains a "chimeric" "toxin" gene comprising a plant promotor sequence derived from the indicated expression vector and a Bt gene cassette.

DETD(216) This example describes the construction of pHD205, an intermediate vector containing a "chimeric" Bt2 "toxin" gene comprising: the nopaline synthase promoter, the Bt2 "toxin" gene cassette from pHD160 and a DNA fragment containing the 3' untranslated region of the nopaline synthase gene including the polyadenylation site. In the "chimeric" gene, the Bt2 gene cassette is oriented such that the expression of the Bt2 protein can be obtained from the. . . are fragments of approximately 6200 bp, 3000 bp, 1800 bp and 920 bp. A recombinant plasmid with the alpha-orientation (the "toxin" gene under the control of the nopaline synthase promotor) is used in subsequent experiments and called pHD205.

DETD(218) This example describes the construction of pHD208. The intermediate vector pHD208 contains a "chimeric" Bt2 "toxin" gene comprising: the promotor from a pea gene encoding a small subunit of ribulose biphosphate carboxylase (Pssu), the Bt2 "toxin" gene cassette from PHD160 and the 3' untranslated region of the octopine synthase gene including the polyadenylation site. The fragments of the "chimeric" gene were assembled in the cloning vector PGV831 as described in this example and as diagrammed in FIG. 29. The. . .

DETD(262) 10. Isolation of plant cells and plants containing the "chimeric" "toxin" gene inserted in their genome

DETD(495) A . . . transformation vectors described herein will contain, stably inserted into their genome, a fragment or newly acquired DNA containing both a "chimeric" Bt "toxin" gene and a marker gene (nos, NPTII). This was confirmed by the results of southern blotting experiments. The new phenotypic traits acquired through this transformation method (expression of Bt "Toxin", antibiotic resistance, nopaline production) will be inherited according to classic Mendelian genetics. To verify stable inheritance of the new traits, F.sub.1 descendants from transformed plants were analysed for the expression of Bt "toxin" and synthesis of nopaline.

DETD(509) TABLE 4

Toxicity of Bt:NPT2 "Fusion" Protein on 3rd InstarP. brassicae (% Mortality After 4 Days)

"Toxin" dose (ug/ml) Bt protein	0.1	0.2	0.3	0.6	1
Bt2	70	NT.sup.(x)			
	90	NT	100		
Bt:NPT2	NT. . .				

DETD(510) TABLE 5

Toxicity of Intact Bt2 Protein, 60 Kd "Processed"Bt2 Protein (Trypsin Digested) and Bt:NPT2 "Fusion" Protein on Larvae of Manduca sexta

% Mortality after 4 days "Toxin" dose: (ng/cm.sup.2)	0	0.67	2	6	18	54	162
130 Kd Bt2	0	0	0	0	3	8. . .	

US PAT NO: 5,840,554 [IMAGE AVAILABLE] L10: 4 of 31

ABSTRACT: Bacillus "thuringiensis" "endotoxin" expression in Pseudomonads can be improved by modifying the gene encoding the Bacillus "thuringiensis" "endotoxin". "Chimeric" genes are created by replacing the segment of the Bacillus "thuringiensis" gene encoding a native protoxin with a segment encoding a different protoxin. Exemplified herein is the cryIF/cryI(b) "chimera" wherein the native cryIF protoxin segment has been substituted by the cryIA(b) protoxin segment, to yield improved expression of the cryIF "toxin" in Pseudomonads. The invention also concerns novel genes and plasmids. BSUM(2) The soil microbe Bacillus "thuringiensis" (B.t.) is a Gram-positive, spore-forming bacterium characterized by parasporal crystalline protein inclusions. These inclusions often appear microscopically as distinctively shaped crystals. The proteins can be highly toxic to pests and specific in their toxic activity. Certain B.t. "toxin" genes have been isolated and sequenced, and recombinant DNA-based B.t. products have been produced and approved for use. In addition,. . . approaches for delivering these B.t. endotoxins to agricultural environments are under development, including the use of plants genetically engineered with "endotoxin" genes for insect resistance and the use of stabilized intact microbial cells as B.t. "endotoxin" delivery vehicles (Gaertner, F. H., L. Kim [1988] TIBTECH 6:S4-S7). Thus, isolated B.t. "endotoxin" genes are becoming commercially valuable.

BSUM(3) Until . . . has been largely restricted to a narrow range of lepidopteran (caterpillar) pests. Preparations of the spores and crystals of B. "thuringiensis" subsp. kurstaki have been used for many years as commercial insecticides for lepidopteran pests. For example, B. "thuringiensis" var. kurstaki HD-1 produces a crystalline .delta.-"endotoxin" which is toxic to the larvae of a number of lepidopteran insects.

BSUM(7) A majority of Bacillus "thuringiensis" .delta.-"endotoxin" crystal protein molecules are composed of two functional segments. The protease-resistant core "toxin" is the first segment and corresponds to about the first half of the protein molecule. The three-dimensional structure of a core segment of a cryIIIA B.t. .delta.-"endotoxin" is known and it is proposed that all related toxins have that same overall structure (Li, J., J. Carroll, D.. . . this second segment will be referred to herein as the "protoxin segment." The protoxin segment is believed to participate in "toxin" crystal formation (Arvidson, H., P. E. Dunn, S. Strand, A. I. Aronson [1989] Molecular Microbiology 3:1533-1534; Choma, C. T., W.. . . K. Surewicz, P. R. Carey, M. Pozsgay, T. Raynor, H. Kaplan [1990] Eur. J. Biochem. 189:523-527). The full 130 kDa "toxin" molecule is rapidly processed to the resistant core segment by protease in the insect gut. The protoxin segment may thus convey a partial insect specificity for the "toxin" by limiting the accessibility of the core to the insect by reducing the protease processing of the "toxin" molecule (Haider, M. Z., B. H. Knowles, D. J. Ellar [1986] Eur. J. Biochem. 156:531-540) or by reducing "toxin" solubility (Aronson, A. I., E. S. Han, W. McGaughey, D. Johnson [1991] Appl. Environ. Microbiol. 57:981-986).

BSUM(8) "Chimeric" proteins joined within the "toxin" domains have been reported between CryIC and CryIA(b) (Honee, G., D. Convents, J. Van Rie, S. Jansens, M. Perferoen, B. Visser [1991] Mol. Microbiol. 5:2799-2806); however, the activity of these "chimeric" proteins was either much less, or undetectable, when compared to CryIC on a relevant insect.

BSUM(9) Honee et al (Honee, G., W. Vriezen, B. Visser [1990] Appl. Environ. Microbiol. 56:823-825) also reported making a "chimeric" "fusion" protein by linking tandem "toxin" domains of CryIC and CryIA(b). The resulting protein had an increased spectrum of activity equivalent to the combined activities of the individual toxins; however, the activity of the "chimeric" was not increased toward any one of the target insects.

BSUM(11) The subject invention concerns the discovery that expression of Bacillus "thuringiensis" (B.t.) .delta.-"endotoxin" in Pseudomonas can be substantially improved by modifying the gene which encodes the B.t. "toxin". Specifically, B.t. "endotoxin" expression in P. fluorescens can be improved by reconstructing the gene so as to replace the native protoxin-encoding segment with an alternate protoxin segment, yielding a "chimeric" gene.

BSUM(12) In specific embodiments of the subject invention, "chimeric" genes can be assembled that substitute a heterologous protoxin segment for a native cryIF protoxin segment. In particular, all or. . . can be used in place of all or part of the region which encodes the protoxin for a native cryIF "toxin". Similarly, a "chimeric" can be constructed wherein the region encoding all or part of the protoxin of a cryIF "toxin" is replaced by D encoding all or part of the protoxin of a cryIA(c)/cryIA(b) "chimeric" gene. In a specific embodiment, the cryIA(c)/cryIA(b) "chimeric" gene is that which has been denoted 436 and which is described in U.S. Pat. No. 5,128,130. This gene can. . .

BSUM(13) The subject invention also includes use of the "chimeric" gene encoding the claimed "toxin". The "chimeric" gene can be introduced into a wide variety of microbial or plant hosts. A transformed host expressing the "chimeric" gene can be used to produce the lepidopteran-active "toxin" of the subject invention. Transformed hosts can be used to produce the insecticidal "toxin" or, in the case of a plant cell transformed to produce the "toxin", the plant will become resistant to insect attack. The subject invention further pertains to the use of the "chimeric" "toxin", or hosts containing the gene encoding the "chimeric" "toxin", in methods for controlling lepidopteran pests.

BSUM(14) Still further, the invention includes the treatment of substantially intact recombinant cells producing the "chimeric" "toxin" of the invention. The cells are treated to prolong the lepidopteran activity when the substantially intact cells are applied to. . . nor diminish the cell's capability of protecting the pesticide. The treated cell acts as a protective coating for the pesticidal "toxin". The "toxin" becomes active upon ingestion by a target insect

DRWDD(5) FIG. 4--The NsiI "toxin"-containing fragment with the new restriction sites is ligated to the vector-containing DNA from pMYC1050.DELTA.BamHI to give pMYC2224. A BamHI-PvuI PCR-derived DNA fragment containing the cryIF "toxin" is exchanged for the equivalent fragment in pMYC2224. The resulting "chimera" is called pMYC2239. B=BamHI, C=ClaI, H=HindIII, N=NsiI, P=PvuI

DRWDD(6) FIG. 5--The small ApaI DNA fragment of pMYC2047 is substituted for the homologous region of pMYC2239 to give plasmid pMYC2244. This "chimera" consists of cryIF in the "toxin" region and cryIA(b) in the protoxin. C=ClaI, H=HindIII, N=NsiI, P=PvuI

DRWDD(9) FIG. 8--A "chimeric" "toxin" containing the 436 protoxin is constructed by substituting a PCR generated PvuI-BstEII protoxin DNA for the homologous fragment in pMYC2523. The. . .

DETD(23) SEQ ID NO. 22 shows the "toxin"-encoding DNA sequence of pMYC2244, which encodes a cryIF/cryIA(b) "chimeric" "toxin".

DETD(24) SEQ ID NO. 23 shows the predicted amino acid sequence of the cryIF/cryIA(b) "chimeric" "toxin" encoded by pMYC2244.

DETD(27) SEQ ID NO. 26 shows the "toxin"-encoding DNA sequence of pMYC2523, which encodes a cryIF/cryIA(b) "chimeric" "toxin" with codon rework.

DETD(29) SEQ ID NO. 28 shows the "toxin"-encoding DNA sequence of pMYC2254, which encodes a cryIF/436 "chimeric" "toxin".

DETD(36) SEQ ID NO. 35 shows the amino acid sequence of a CryIF/CryIA(b) "chimeric" "toxin" of the subject invention that corresponds to the "Cons" sequence shown in FIG. 9.

DETD(37) SEQ ID NO. 36 shows the amino acid sequence of a "chimeric" "toxin" of the subject invention that incorporates the alternative amino acids as shown in the first "Alt" sequence listed above the. . .

DETD(38) SEQ ID NO. 37 shows the amino acid sequence of a "chimeric" "toxin" of the subject invention that incorporates the alternative amino acids as shown in the second "Alt" sequence listed above the. . .

DETD(39) SEQ ID NO. 38 shows the amino acid sequence of a "chimeric" "toxin" of the subject invention that incorporates the alternative amino acids as shown in the third "Alt" sequence listed above the. . .

DETD(41) The subject invention concerns the discovery that certain "chimeric" genes encoding B.t. toxins have improved expression in recombinant Pseudomonas fluorescens. The "chimeric" genes encode toxins wherein all or part of the native protoxin portion has been replaced with all or part of the protoxin from another B.t. "toxin". Specifically exemplified herein are genes which encode a B.t. "toxin" which consists essentially of a cryIF core N-terminal "toxin" portion attached to a protoxin segment which is derived from either a cryIA(b) "toxin" or a cryIA(c)/cryIA(b) "toxin" as described herein. As used herein, reference to a "core" "toxin" portion refers to the portion of the full length B.t. "toxin", other than the protoxin, which is responsible for the pesticidal activity of the "toxin".

DETD(45) The . . . can be carried out according to the subject invention. BamHI and PvuI cloning sites can be introduced into a cryIA(c)/cryIA(b) "chimeric" "toxin" gene by mutagenesis using the PCR technique of Splice Overlap Extension (SOE) (Horton, R. M., H. D. Hunt, S. N.. . . pMYC2224. The new plasmid, which we designated pMYC2239, consisted of a short segment of cryIA(c) followed by cryIF to the "toxin"/protoxin segment junction. Thus, the protoxin segment was now derived from cryIA(b) (pMYC1050). An ApaI fragment derived from the cryIF clone. . . substituted for the ApaI fragment in pMYC2239. The resulting clone (pMYC2244) consisted of cryIF from the initiator methionine to the "toxin"/protoxin segment junction and cryIA(b) to the end of the coding region. Clone pMYC2243 was constructed by SOE to introduce silent. . . from pMYC2243 that contained the silent changes was substituted for the ApaI fragment in pMYC2244 to give clone pMYC2523. The "chimeric" pMYC2523 showed an expression improvement over pMYC2243, which contains unchanged cryIF protein sequence.

DETD(47) The "chimeric" toxins of the subject invention comprise a full core N-terminal "toxin" portion of a B.t. "toxin" and, at some point past the end of the "toxin" portion, the protein has a transition to a heterologous protoxin sequence. The transition to the heterologous protoxin segment can occur at approximately the "toxin"/protoxin junction or, in the alternative, a portion of the native protoxin (extending past the "toxin" portion) can be retained with the transition to the heterologous protoxin occurring downstream. As an example, one "chimeric" "toxin" of the subject invention has the full "toxin" portion of cryIF (amino acids 1-601) and a heterologous protoxin (amino acids 602 to the C-terminus). In a preferred embodiment, the heterologous portion of the protoxin is derived from a cryIA(b) or 436 "toxin".

DETD(48) A . . . certain class such as cryIF, will vary to some extent in length and the precise location of the transition from "toxin" portion to protoxin portion. Typically, the cryIA(b) and cryIF toxins are about 1150 to about 1200 amino acids in length. The transition from "toxin" portion to protoxin portion will typically occur at between about 50% to about 60% of the full length "toxin". The "chimeric" "toxin" of the subject invention will include the full expanse of this core N-terminal "toxin" portion. Thus, the "chimeric" "toxin" will comprise at least about 50% of the full length cryIF B.t. "toxin". This will typically be at least about 590 amino acids. With regard to the protoxin portion, the full expanse of the cryIA(b) protoxin portion extends from the end of the "toxin" portion to the C-terminus of the molecule. It is the last about 100 to 150 amino acids of this portion which are most critical to include in the "chimeric" "toxin" of the subject invention. In a "chimeric" "toxin" specifically exemplified herein, at least amino acids 1043 (of SEQ ID NO. 23) to the C-terminus of the cryIA(b) molecule. . . marks the location in the protoxin segment of the molecule beyond which heterologous amino acids will always occur in the "chimeric" "toxin". In another example, the peptide shown as SEQ ID NO. 31 occurs at amino acids 1061 to 1068. In this. . . approximately 5 to 10% of the overall B.t. protein which should comprise heterologous DNA (compared to the cryIF core N-terminal "toxin" portion) in the "chimeric" "toxin" of the subject invention. In the specific examples contained herein, heterologous protoxin sequences occur from amino acid 640 to the. . .

DETD(49) Thus, a preferred embodiment of the subject invention is a "chimeric" B.t. "toxin" of about 1150 to about 1200 amino acids in length, wherein the "chimeric" "toxin" comprises a cryIF core N-terminal "toxin" portion of at least about 50 to 60% of a full cryIF molecule, but no more than about 90 to 95% of the full molecule. The "chimeric" "toxin" further comprises a cryIA(b) or a 436 protoxin C-terminal portion which comprises at least about 5 to 10% of the. . . transition from cryIF to cryIA(b) or 436 sequence thus occurs within the protoxin segment (or at the junction of the "toxin" and protoxin segments) between about 50% and about 95% of the way through the molecule. In the specific examples provided. . .

DETD(50) A specific embodiment of the subject invention is the "chimeric" "toxin" shown in FIG. 9. Other constructs may be made and used by those skilled in this art having the benefit of the teachings provided herein. The core "toxin" segment of cryI proteins characteristically ends with the sequence: Val/Leu Tyr/Ile Ile Asp Arg/Lys Ile/Phe Glu Ile/Phe/Leu Ile/Leu/Val Pro/Leu Ala/Val. . . NO. 23. Additionally, the protoxin segments of the cryI toxins (which follow residue 601) bear more sequence similarity than the "toxin" segments. Because of this sequence similarity, the transition point in the protoxin segment for making a "chimeric" protein between the cryIF sequence and the cryIA(b) or 436 sequence can be readily determined by one skilled in the. . .

DETD(51) Therefore a "chimeric" "toxin" of the subject invention can comprise the full cryIF "toxin" and a portion of the cryIF protoxin, transitioning to the corresponding cryIA(b) or 436 sequence at any position between the end of the "toxin" segment (as defined above) and the end of the peptide sequence shown in SEQ ID NO. 31. Preferably, the amino acid sequence of the C-terminus of the "chimeric" "toxin" comprises a cryIA(b) sequence or a sequence from the 436 gene or an equivalent of one of these sequences.

DETD(53) FIG. . . . used in the toxins of the subject invention. SEQ ID NO. 35 shows the amino acid sequence of a CryIF/CryIA(b) "chimeric" "toxin" of the subject invention that corresponds to the "Cons" sequence shown in FIG. 9. SEQ ID NO. 36 shows the amino acid sequence of a "chimeric" "toxin" of the subject invention that incorporates the alternative amino acids as shown in the first "Alt" sequence listed above the "Cons" sequence shown in FIG. 9. SEQ ID NO. 37 shows the amino acid sequence of a "chimeric" "toxin" of the subject invention that incorporates the alternative amino acids as shown in the second "Alt" sequence listed above the first "Alt" sequence shown in FIG. 9. SEQ ID NO. 38 shows the amino acid sequence of a "chimeric" "toxin" of the subject invention that incorporates the alternative amino acids as shown in the third "Alt" sequence listed above the. . . shown in FIG. 9. It is also well known in the art that various mutations can be made in a "toxin" sequence without changing the activity of a "toxin". Furthermore, due to the degeneracy of the genetic code, a variety of DNA sequences can be used to encode a particular "toxin". These alternative DNA and amino acid sequences can be used according to the subject invention by a person skilled in. . .

DETD(55) The subject invention not only includes the novel "chimeric" toxins and the genes encoding these toxins but also includes uses of these novel toxins and genes. For example, a. . . of the subject invention may be used to transform host cells. These host cells expressing the gene and producing the "chimeric" "toxin" may be used in insecticidal compositions or, in the case of a transformed plant cell, in conferring insect resistance to. . .

DETD(60) A . . . for determining in a known manner whether hybridization has occurred. Such a probe analysis provides a rapid method for identifying "toxin"-encoding genes of the subject invention. Preferably, such genes would be cryIF genes whose core "toxin"-encoding portions can then be used with a cryIA(b) or 436 protoxin-encoding portion to create a "chimeric" gene according to the subject invention. The nucleotide segments which are used as probes according to the invention can be. . .

DETD(61) Certain "chimeric" toxins of the subject invention have been specifically exemplified herein. It should be readily apparent that the subject invention comprises. . . variant or equivalent toxins (and nucleotide sequences encoding equivalent toxins) having the same or similar pesticidal activity of the exemplified "toxin". Equivalent toxins will have amino acid homology with the exemplified "toxin". This amino acid homology will typically be greater than 75%, preferably be greater than 90%, and most preferably be greater than 95%. The amino acid homology will be highest in critical regions of the "toxin" which account for biological activity or are involved in the determination of three-dimensional configuration which ultimately is responsible for the. . .

DETD(65) A gene encoding a "chimeric" "toxin" of the subject invention can be introduced into a wide variety of microbial or plant hosts. Expression of the "toxin" gene results, directly or indirectly, in the intracellular production and maintenance of the pesticidal "chimeric" "toxin". With suitable microbial hosts, e.g., Pseudomonas, the microbes can be applied to the situs of the pest, where they will proliferate and be ingested. The result is control of the pest. Alternatively, the microbe hosting the "toxin" gene can be treated under conditions that prolong the activity of the "toxin" and stabilize the cell. The treated cell, which retains the toxic activity, then can be applied to the environment of. . .

DETD(66) Where the gene encoding the "chimeric" "toxin" is introduced via a suitable vector into a microbial host, and said host is applied to the environment in a. . .

DETD(68) A wide variety of ways are available for introducing a gene encoding a "chimeric" "toxin" into a microorganism host under conditions which allow for the stable maintenance and expression of the gene. These methods are. . .

DETD(70) As mentioned above, recombinant cells producing the "chimeric" "toxin" of the subject invention can be treated to prolong the toxic activity and stabilize the cell. The pesticide microcapsule that is formed comprises the B.t. "toxin" within a cellular structure that has been stabilized and will protect the "toxin" when the microcapsule is applied to the environment of the target pest. Suitable host cells may include either prokaryotes or. . .

DETD(72) Treatment of the microbial cell, e.g., a microbe containing the gene encoding a "chimeric" "toxin" of the subject invention, can be by chemical or physical means, or by a combination of chemical and/or physical means, so long as the technique does not deleteriously affect the properties of the "toxin", nor diminish the cellular capability of protecting the "toxin". Examples of chemical reagents are halogenating agents, particularly halogens of atomic no. 17-80. More particularly, iodine can be used under. . . and Company, 1967); or a combination of physical (heat) and chemical agents that preserve and prolong the activity of the "toxin" produced in the cell when the cell is administered to the host environment. Examples of physical means are short wavelength. . .

DETD(76) The cellular host containing the gene encoding a "chimeric" "toxin" of the subject invention may be grown in any convenient nutrient medium, where the DNA construct provides a selective advantage,. . .

DETD(78) Recombinant microbes comprising a gene encoding a "chimeric" "toxin" disclosed herein, can be formulated into bait granules and applied to the soil. Formulated product can also be applied as. . .

DETD(95) A . . . be found in EPO patent application 0 471 564. A cryIA(c)/cryIA(b) gene, referred to herein as the 436 gene and "toxin", are described in U.S. Pat. No. 5,055,294. A plasmid designated pMYC1050 contains a cryIA(c)/cryIA(b) "chimeric" gene known as the 420 gene. pMYC1050 was constructed by re-cloning the "toxin" gene and promoter of pM3,130-7 (disclosed in U.S. Pat. No. 5,055,294) into a pTJS260-based vector such as pMYC467 (disclosed in U.S. Pat. No. 5,169,760) by methods well known in the art. In particular, the pM3,130-7 promoter and "toxin" gene can be obtained as a BamHI to NdeI fragment and placed into the pMYC467 plasmid replacing a fragment bounded. . .

DETD(120) A "toxin"-containing DNA fragment was generated by PCR with primers L/D on template pMYC1260. The DNA was digested with BglII and PvuI. . . correct plasmids were identified by PCR analysis and agarose-TBE gel electrophoresis using the primer set N/O, which bridges the BamHI/BglII "fusion" junction.

DETD(162) A second type of "chimeric" "toxin" was assembled by substituting the 436 protoxin module for the cryIA(b) protoxin in pMYC2523 (FIG. 8). The 436 protoxin sequence. . .

DETD(170) Insertion of the Gene Encoding the "Chimeric" "Toxin" Into Plants

DETD(172) The gene encoding the "chimeric" "toxin", as disclosed herein, can be inserted into plant cells using a variety of techniques which are well known in the. . . higher plants. The vectors comprise, for example, pBR322, pUC series, M13mp series, pACYC184, etc. Accordingly, the sequence encoding the B.t. "toxin" can be inserted into the vector at a suitable restriction site. The resulting plasmid is used for transformation into E.. . .

DETD(179) Cloning of the Gene Encoding the "Chimeric" "Toxin" Into Insect Viruses

DETD(180) A . . . genome of the insect virus, thus enhancing the pathogenicity of the virus. Methods for constructing insect viruses which comprise the "chimeric" "toxin" gene are well known and readily practiced by those skilled in the art. These procedures are described, for example, in. . .

We claim:

1. A method for improving Bacillus "thuringiensis" .delta.-"endotoxin" expression in a Pseudomonad comprising transforming said Pseudomonad with a gene encoding a Bacillus "thuringiensis" "toxin" wherein said Bacillus "thuringiensis" "toxin" is a "chimeric" "toxin" comprising a cryIF core N-terminal "toxin" portion and a C-terminal protoxin portion from a cryIA(b) "toxin" or a cryIA(c)/cryIA(b) "chimeric" "toxin".

CLMS(3) 3. The method, according to claim 1, wherein said Pseudomonad is transformed with a nucleotide sequence encoding a "chimeric" Bacillus "thuringiensis" "toxin" of approximately 1150 to 1200 amino acids, wherein said "toxin" comprises a cryIF core N-terminal sequence of at least about 590 amino acids and no more than about 1100 amino. . . acids, and wherein said cryIA(b) or cryIA(c)/cryIA(b) protoxin portion comprises at least 100 amino acids at the C-terminus of said "toxin".

CLMS(9) 9. The method, according to claim 1, wherein said heterologous protoxin portion is that of a cryIA(c)/cryIA(b) "chimeric" "toxin". CLAIMS:

CLMS(20) 20. Treated, substantially intact cells containing an intracellular "toxin", which "toxin" is a result of expression of a Bacillus "thuringiensis" gene encoding a "toxin" active against lepidopteran pests wherein said "toxin" is a "chimeric" "toxin" comprising a cryIF core N-terminal "toxin" portion and a protoxin portion from a cryIA(b) or a cryIA(c)/cryIA(b)"chimeric" "toxin", wherein said cells are treated by chemical or physical means to prolong the insecticidal activity when said cells are applied: . .

CLMS(21) 21. A process for controlling lepidopteran pests comprising contacting said pest with a lepidopteran-controlling effective amount of a substantially pure "chimeric" Bacillus "thuringiensis" "toxin" comprising a cryIF core N-terminal "toxin" portion and a C-terminal protoxin portion from a cryIA(b) "toxin" or cryIA(b)/cryIA(c) "chimeric" "toxin".

US PAT NO: 5,827,514 [IMAGE AVAILABLE] L10: 5 of 31

ABSTRACT: Disclosed are compositions and processes for controlling lepidopteran pests. These compositions comprise synergistic combinations of a CryIF "chimeric" and CryIA(c) "chimeric" Bacillus "thuringiensis" .delta.-"endotoxin". These compositions have been found to exhibit excellent activity against lepidopteran pests.

BSUM(2) The soil microbe Bacillus "thuringiensis" (B.t.) is a Gram-positive, spore-forming bacterium characterized by parasporal crystalline protein inclusions. These inclusions often appear microscopically as distinctively shaped crystals. The proteins can be highly toxic to pests and specific in their toxic activity. Certain B.t. "toxin" genes have been isolated and sequenced, and recombinant DNA-based B.t. products have been produced and approved for use. In addition,. . . approaches for delivering these B.t. endotoxins to agricultural environments are under development, including the use of plants genetically engineered with "endotoxin" genes for insect resistance and the use of stabilized intact microbial cells as B.t. "endotoxin" delivery vehicles (Gaertner, F. H., L. Kim [1988] TIBTECH 6:S4-S7). Thus, isolated B.t. "endotoxin" genes are becoming commercially valuable.

BSUM(3) Until . . . has been largely restricted to a narrow range of lepidopteran (caterpillar) pests. Preparations of the spores and crystals of B. "thuringiensis" subsp. kurstaki have been used for many years as commercial insecticides for lepidopteran pests. For example, B. "thuringiensis" var. kurstaki HD-1 produces a crystal called a .delta.-"endotoxin" which is toxic to the larvae of a number of lepidopteran insects.

BSUM(8) A majority of Bacillus "thuringiensis" .delta.-"endotoxin" crystal protein molecules are composed of two functional segments. The protease-resistant core "toxin" is the first segment and corresponds to about the first half of the protein molecule. The three-dimensional structure of a core segment of a cryIIIA B.t. .delta.-"endotoxin" is known and it is proposed that all related toxins have that same overall structure (Li, J., J. Carroll, D.. . . this second segment will be referred to herein as the "protoxin segment." The protoxin segment is believed to participate in "toxin" crystal formation (Arvidson, H., P. E. Dunn, S. Strand, A. I. Aronson [1989] Molecular Microbiology 3:1533-1534; Choma, C. T., W.. . . K. Surewicz, P. R. Carey, M. Pozsgay, T. Raynor, H. Kaplan [1990] Eur. J. Biochem. 189:523-527). The full 130 kDa "toxin" molecule is rapidly processed to the protease resistant core segment by protease in the insect gut. The protoxin segment may thus convey a partial insect specificity for the "toxin" by limiting the accessibility of the core to the insect by reducing the protease processing of the "toxin" molecule (Haider, M. Z., B. H. Knowles, D. J. Ellar [1986] Eur. J. Biochem. 156:531-540) or by reducing "toxin" solubility (Aronson, A. I., E. S. Han, W. McGaughey, D. Johnson [1991] Appl. Environ. Microbiol. 57:981-986).

BSUM(9) "Chimeric" proteins joined within the "toxin" domains have been reported between CryIC and CryIA(b) (Honee, G., D. Convents, J. Van Rie, S. Jansens, M. Perferoen, B. Visser [1991] Mol. Microbiol. 5:2799-2806); however, the activity of these "chimeric" proteins was either much less, or undetectable, when compared to CryIC on a relevant insect.

BSUM(10) Honee et al. (Honee, G., W. Vriezen, B. Visser [1990] Appl. Environ. Microbiol. 56:823-825) also reported making a "chimeric" "fusion" protein by linking tandem "toxin" domains of CryIC and CryIA(b). The resulting protein had an increased spectrum of activity equivalent to the combined activities of the individual toxins; however, the activity of the "chimeric" was not increased toward any one of the target insects.

BSUM(14) The subject invention concerns the discovery of advantageous increased activity against lepidopteran pests achieved by the combination of two Bacillus "thuringiensis" (B.t.) .delta.-"endotoxin" proteins. More specifically, a CryIF "chimeric" "toxin" combined with a CryIA(c) "chimeric" "toxin" act in synergy to yield unexpected enhanced toxicity to lepidopteran pests.

BSUM(16) "Chimeric" CryIF genes useful according to the subject invention can be assembled that substitute a heterologous protoxin segment for all or. . . can be used in place of all or part of the region which encodes the protoxin for a native cryIF "toxin". Similarly, a "chimeric" gene can be constructed wherein the region encoding all or part of the protoxin of a cryIF "toxin" is replaced by DNA encoding all or part of the protoxin of a

cryIA(c)/cryIA(b) "chimeric" gene. In a specific embodiment, the cryIA(c)/cryIA(b) "chimeric" gene is that which has been denoted 436 and which is described in U.S. Pat. No. 5,128,130. This gene can. . . DRAWING DESC:

DRWDD(5) FIG. 4 The NsiI "toxin"-containing fragment with the new restriction sites is ligated to the vector-containing DNA from pMYC1050.DELTA.BamHI to give pMYC2244. A BamHI-PvuI PCR-derived DNA fragment containing the cryIF "toxin" is exchanged for the equivalent fragment in pMYC2244. The resulting "chimera" is called pMYC2239. B=BamHI, C=ClaI, H=HindIII, N=NsiI, P=PvuI

DRWDD(6) FIG. . . . The small ApaI DNA fragment of pMYC2047 is substituted for the homologous region of pMYC2239 to give plasmid pMYC2244. This "chimera" consists of cryIF in the "toxin" region and cryIA(b) in the protoxin. C=ClaI, H=HindIII, N=NsiI, P=PvuI

DRWDD(9) FIG. 8 A "chimeric" "toxin" containing the 436 protoxin is constructed by substituting a PCR-generated PvuI-BstEII protoxin DNA for the homologous fragment in pMYC2523. The. . .

DETD(23) SEQ ID NO. 22 shows the "toxin"-encoding DNA sequence of pMYC2244, which encodes a cryIF/cryIA(b) "chimeric" "toxin".

DETD(24) SEQ ID NO. 23 shows the predicted amino acid sequence of the cryIF/cryIA(b) "chimeric" "toxin" encoded by pMYC2244.

DETD(27) SEQ ID NO. 26 shows the "toxin"-encoding DNA sequence of pMYC2523, which encodes a cryIF/cryIA(b) "chimeric" "toxin" with codon rework.

DETD(29) SEQ ID NO. 28 shows the "toxin"-encoding DNA sequence of pMYC2254, which encodes a cryIF/436 "chimeric" "toxin".

DETD(36) SEQ ID NO. 35 shows the amino acid sequence of a CryIF/CryIA(b) "chimeric" "toxin" of the subject invention that corresponds to the "Cons" sequence shown in FIG. 9.

DETD(37) SEQ ID NO. 36 shows the amino acid sequence of a "chimeric" "toxin" of the subject invention that incorporates the alternative amino acids as shown in the first "Alt" sequence listed above the. . .

DETD(38) SEQ ID NO. 37 shows the amino acid sequence of a "chimeric" "toxin" of the subject invention that incorporates the alternative amino acids as shown in the second "Alt" sequence listed above the. . .

DETD(39) SEQ ID NO. 38 shows the amino acid sequence of a "chimeric" "toxin" of the subject invention that incorporates the alternative amino acids as shown in the third "Alt" sequence listed above the. . .

DETD(41) The subject invention concerns the unexpected enhanced pesticidal activity resulting from the combination of a CryIF "chimeric" "toxin" and a CryIA(c) "chimeric" "toxin". The combination surprisingly has increased activity against lepidopteran pests. Preparations of combinations of isolates that produce the two "chimeric" toxins can be used to practice the subject invention. Pseudomonas fluorescens cells transformed with B.t. genes can serve as one. . . of the toxins of the subject invention. For example, a lactose-inducible P. fluorescens strain comprising a gene encoding a CryIF/CryIA(b) "toxin", and P. fluorescens MR436, which comprises a gene encoding a CryIA(c)/CryIA(b) "chimeric" "toxin", can be used to practice the subject invention. These two Pseudomonas strains can be combined in a physical blend that. . .

DETD(45) In accordance with the subject invention, it has been discovered that products comprising the two "chimeric" toxins have been discovered to require a lower total protein content for product application, thus providing the user greater economy. Insects which are less susceptible to the action of a single "toxin" will be more greatly affected by the combination of toxins of the subject invention, rendering a product containing the two toxins more efficacious than products containing a single "toxin". Additionally, pests are less likely to develop a rapid resistance to a product containing the two toxins, than to products containing a single "toxin".

DETD(47) The "chimeric" toxins of the subject invention comprise a full core N-terminal "toxin" portion of a B.t. "toxin" and, at some point past the end of the "toxin" portion, the protein has a transition to a heterologous protoxin sequence. The N-terminal "toxin" portion of a B.t. "toxin" is refererred to herein as the "core" "toxin". The transition to the heterologous protoxin segment can occur at approximately the "toxin"/protoxin junction or, in the alternative, a portion of the native protoxin (extending past the "toxin" portion) can be retained with the transition to the heterologous protoxin occurring downstream. As an example, one "chimeric" "toxin" of the subject invention has the full "toxin" portion of cryIF (amino acids 1-601) and a heterologous protoxin (amino acids 602 to the C-terminus). In a
preferred embodiment, the heterologous portion of the protoxin is derived
from a cryIA(b) or 436 "toxin".

DETD(48) A . . . certain class such as cryIF, will vary to some extent in length and the precise location of the transition from "toxin" portion to protoxin portion. Typically, the cryIA(b) and cryIF toxins are about 1150 to about 1200 amino acids in length. The transition from "toxin" portion to protoxin portion will typically occur at between about 50% to about 60% of the full length "toxin". The "chimeric" "toxin" of the subject invention will include the full expanse of this core N-terminal "toxin" portion. Thus, the "chimeric" "toxin" will comprise at least about 50% of the full length cryIF B.t. "toxin". This will typically be at least about 590 amino acids. With regard to the protoxin portion, the full expanse of the cryIA(b) protoxin portion extends from the end of the "toxin" portion to the C-terminus of the molecule. It is the last about 100 to 150 amino acids of this portion which are most critical to include in the "chimeric" "toxin" of the subject invention. In a "chimeric" "toxin" specifically exemplified herein, at least amino acids 1043 (of SEQ ID NO. 23) to the C-terminus of the cryIA(b) molecule. . . marks the location in the protoxin segment of the molecule beyond which heterologous amino acids will always occur in the "chimeric" "toxin". In another example, the peptide shown as SEQ ID NO. 31 occurs at amino acids 1061 to 1068. In this. . . approximately 5 to 10% of the overall B.t. protein which should comprise heterologous DNA (compared to the cryIF core N-terminal "toxin" portion) in the "chimeric" "toxin" of the subject invention. In the specific examples contained herein, heterologous protoxin sequences occur from amino acid 640 to the. . .

DETD(49) Thus, a preferred embodiment of the subject invention is a "chimeric" B.t. "toxin" of about 1150 to about 1200 amino acids in length, wherein the "chimeric" "toxin" comprises a cryIF core N-terminal "toxin" portion of at least about 50 to 60% of a full cryIF molecule, but no more than about 90 to 95% of the full molecule. The "chimeric" "toxin" further comprises a cryIA(b) or a 436 protoxin C-terminal portion which comprises at least about 5 to 10% of the. . . transition from cryIF to cryIA(b) or 436 sequence thus occurs within the protoxin segment (or at the junction of the "toxin" and protoxin segments) between about 50% and about 95% of the way through the molecule. In the specific examples provided. . .

DETD(50) A specific embodiment of the subject invention is the "chimeric" "toxin" shown in FIG. 9. Other constructs may be made and used by those skilled in this art having the benefit of the teachings provided herein. The core "toxin" segment of cryI proteins characteristically ends with the sequence: Val/Leu Tyr/Ile Ile Asp Arg/Lys Ile/Phe Glu Ile/Phe/Leu Ile/Leu/Val Pro/Leu Ala/Val. . . NO. 23. Additionally, the protoxin segments of the cryI toxins (which follow residue 601) bear more sequence similarity than the "toxin" segments. Because of this sequence similarity, the transition point in the protoxin segment for making a "chimeric" protein between the cryIF sequence and the cryIA(b) or 436 sequence can be readily determined by one skilled in the. . .

DETD(51) Therefore a "chimeric" "toxin" of the subject invention can comprise the full cryIF "toxin" and a portion of the cryIF protoxin, transitioning to the corresponding cryIA(b) or 436 sequence at any position between the end of the "toxin" segment (as defined above) and the end of the peptide sequence shown in SEQ ID NO. 31. Preferably, the amino acid sequence of the C-terminus of the "chimeric" "toxin" comprises a cryIA(b) sequence or a sequence from the 436 gene or an equivalent of one of these sequences.

DETD(53) FIG. . . . used in the toxins of the subject invention. SEQ ID NO. 35 shows the amino acid sequence of a CryIF/CryIA(b) "chimeric" "toxin" of the subject invention that corresponds to the "Cons" sequence shown in FIG. 9. SEQ ID NO. 36 shows the amino acid sequence of a "chimeric" "toxin" of the subject invention that incorporates the alternative amino acids as shown in the first "Alt" sequence listed above the "Cons" sequence shown in FIG. 9. SEQ ID NO. 37 shows the amino acid sequence of a "chimeric" "toxin" of the subject invention that incorporates the alternative amino acids as shown in the second "Alt" sequence listed above the first "Alt" sequence shown in FIG. 9. SEQ ID NO. 38 shows the amino acid sequence of a "chimeric" "toxin" of the subject invention that incorporates the alternative amino acids as shown in the third "Alt" sequence listed above the. . . shown in FIG. 9. It is also well known in the art that various mutations can be made in a "toxin" sequence without changing the activity of a "toxin". Furthermore, due to the degeneracy of the genetic code, a variety of DNA sequences can be used to encode a particular "toxin". These alternative DNA and amino acid sequences can be used according to the subject invention by a person skilled in. . .

DETD(55) The . . . can be carried out according to the subject invention. BamHI and PvuI cloning sites can be introduced into a cryIA(c)/cryIA(b) "chimeric" "toxin" gene by mutagenesis using the PCR technique of Splice Overlap Extension (SOE) (Horton, R. M., H. D. Hunt, S. N.. . . pMYC2224. The new plasmid, which we designated pMYC2239, consisted of a short segment of cryIA(c) followed by cryIF to the "toxin"/protoxin segment junction. Thus, the protoxin segment was now derived from cryIA(b) (pMYC1050). An ApaI fragment derived from the cryIF clone. . . substituted for the ApaI fragment in pMYC2239. The resulting clone (pMYC2244) consisted of cryIF from the initiator methionine to the "toxin"/protoxin segment junction and cryIA(b) to the end of the coding region. Clone pMYC2243 was constructed by SOE to introduce silent. . . from pMYC2243 that contained the silent changes was substituted for the ApaI fragment in pMYC2244 to give clone pMYC2523. The "chimeric" pMYC2523 showed an expression improvement over pMYC2243, which contains unchanged cryIF protein sequence.

DETD(68) Treatment of cells. Bacillus "thuringiensis" or recombinant cells expressing the B.t. toxins can be treated to prolong the "toxin" activity and stabilize the cell. The pesticide microcapsule that is formed comprises the B.t. "toxin" or toxins within a cellular structure that has been stabilized and will protect the "toxin" when the microcapsule is applied to the environment of the target pest. Suitable host cells may include either prokaryotes or. . .

DETD(116) A "toxin"-containing DNA fragment was generated by PCR with primers L/D on template pMYC1260. The DNA was digested with BglII and PvuI. . . correct plasmids were identified by PCR analysis and agarose-TBE gel electrophoresis using the primer set N/O, which bridges the BamHI/BglII "fusion" junction.

DETD(151) A second type of "chimeric" "toxin" was assembled by substituting the 436 protoxin module for the cryIA(b) protoxin in pMYC2523 (FIG. 8). The 436 protoxin sequence. . .

DETD(159) Analysis for Synergy Between CryIF "Chimeric" "Toxin" and CryIA(c) "Chimeric" "Toxin" Against the Corn Earworm, Heliothis zea

DETD(170) TABLE 2

% INHIBITION "chimeric" toxins .mu.g "toxin"/g diet	cryIF/ a	cryIA(c)/ b	1:1 mix of the two E(exp)	E(obs)	SF	Rate	cryIA(b)	cryIA(b)
50.0	--	--	50	78	1.6	25.0	13	23
22 62	2.8. . .							

DETD(172) Analysis for Synergy Between CryIF "Chimeric" "Toxin" and CryIA(c) "Chimeric" "Toxin" Against the Corn Earworm, Heliothis zea

We claim:

1. A composition for controlling lepidopteran pests, wherein said composition comprises cells which express a CryIF "chimeric" core "toxin"-containing protein and a CryIA(c) "chimeric" core "toxin"-containing protein.

CLMS(2) 2. The composition, according to claim 1, comprising a cell expressing a CryIF "chimeric" core "toxin"-containing protein and a cell expressing a CryIA(c) "chimeric" core "toxin"-containing protein.

CLMS(3) 3. The composition, according to claim 1, comprising a cell expressing a CryIF "chimeric" core "toxin"-containing protein and a CryIA(c) "chimeric" core "toxin"-containing protein.

CLMS(4) 4. The composition, according to claim 1, wherein said CryIF "chimeric" core "toxin"-containing protein comprises a CryIF core N-terminal protein portion and a heterologous C-terminal "toxin" portion from a CryIA(b) "toxin" or CryIA(b)/CryIA(c) "chimeric" "toxin".

CLMS(5) 5. The composition, according to claim 4, wherein said CryIF "chimeric" core "toxin"-containing protein has approximately 1150 to 1200 amino acids and comprises a CryIF core N-terminal sequence of at least about 590. . .

CLMS(10) 10. The composition, according to claim 6, wherein said CryIF "chimeric" core "toxin"-containing protein comprises an amino acid sequence selected from the group consisting of SEQ ID NO. 35, SEQ ID NO. 36,. . .

CLMS(11) 11. The composition, according to claim 1, wherein said CryIA(c) "chimeric" core "toxin"-containing protein has an amino acid sequence shown in SEQ ID NO. 34.

CLMS(14) 14. A host transformed to express both a CryIF "chimeric" core "toxin"-containing protein and a CryIA(c) "chimeric" core "toxin"-containing protein, wherein said host is a microorganism or a plant cell.

CLMS(15) 15. . . . pests, or the environment of said pests, with an effective amount of a composition comprising cells which produce a CryIF "chimeric" core "toxin"-containing protein and a CryIA(c) "chimeric" core "toxin"-containing protein.

CLMS(16) 16. The method, according to claim 15, wherein said composition comprises a cell expressing a CryIF "chimeric" core "toxin"-containing protein and a cell expressing a CryIA(c) "chimeric" core "toxin"-containing protein.

CLMS(17) 17. The method, according to claim 15, wherein said composition comprises a cell expressing a CryIF "chimeric" core "toxin"-containing protein and a CryIA(c) "chimeric" core "toxin"-containing protein.

CLMS(18) 18. The method, according to claim 15, wherein said CryIF "chimeric" core "toxin"-containing protein comprises a CryIF core N-terminal "toxin" portion and a heterologous C-terminal protoxin portion from a Cry "toxin" or CryIA(b)/CryIA(c) "chimeric" "toxin".

CLMS(19) 19. The method, according to claim 18, wherein said CryIF "chimeric" core "toxin"-containing protein has approximately 1150 to 1200 amino acids and comprises a CryIF core N-terminal sequence of at least about 590. . .

CLMS(24) 24. The method, according to claim 20, wherein said CryIF "chimeric" core "toxin"-containing protein comprises an amino acid sequence selected from the group consisting of SEQ ID NO. 35, SEQ ID NO. 36,. . .

CLMS(25) 25. The method, according to claim 18, wherein said CryIA(c) "chimeric" core "toxin"-containing protein has an amino acid sequence shown in SEQ ID NO. 34.

US PAT NO: 5,824,302 [IMAGE AVAILABLE] L10: 6 of 31

ABSTRACT: According . . . an insecticidal amount of a transgenic Zea mays plant that expresses a polypeptide having the insect toxicity properties of Bacillus "thuringiensis" crystal protein.

BSUM(12) Bacillus "thuringiensis" (hereinafter Bt) is a species of bacteria that produces a crystal protein, also referred to as delta-"endotoxin". This crystal protein is, technically, a protoxin that is converted into a "toxin" upon being ingested by larvae of lepidopteran, coleopteran and dipteran insects.

BSUM(32) This invention further provides "chimeric" genes capable of expressing in corn cells a polypeptide having substantially the insect toxicity properties of Bt crystal protein (hereinafter, "chimeric" Bt "toxin" gene).

BSUM(33) Additional embodiments of the present invention include the "chimeric" Bt "toxin" gene in vectors, bacteria, plant cells in culture, and plant cells in living plants, as well as methods for producing a "toxin" having substantially the insect toxicity properties of Bt crystal protein in corn cells and methods for controlling or killing insects by feeding them corn cells containing a gene that expresses such a "toxin".

BSUM(51) It . . . provide a method of controlling insect larvae, preferably lepidopteran, coleopteran and dipteran larvae by feeding them corn plant cells containing "chimeric" genes which express an insecticidal amount of a Bt crystal "toxin" or a "toxin" having substantially the insect toxicity properties of Bt.alpha..beta. crystal protein

DRWDD(23) FIG. 13 shows the nucleotide sequence of the "endotoxin" gene from Bacillus "thuringiensis" var. kurstaki HD1. A preferred sequence of nucleotides that codes for a crystal protein is that shown as nucleotides 156. . .

DETD(141) The present invention is directed to the production of a "chimeric" Bt "toxin" gene. The corn plant cells contemplated include cells from all genotypes (varieties, cultivars, inbred lines, hybrids, etc.) of corn plants. . .

DETD(159) The coding region of the "chimeric" gene contains a nucleotide sequence that codes for a polypeptide having substantially the toxicity properties of a Bt delta-"endotoxin" crystal protein. A polypeptide, for the purpose of the present invention, has substantially the toxicity properties of Bt delta-"endotoxin" crystal protein if it is insecticidal to a similar range of insect larvae as is the crystal protein from a. . .

DETD(161) The coding sequence of the "chimeric" gene may also code for a polypeptide that differs from a naturally occurring crystal protein delta-"endotoxin" but that still has substantially the insect toxicity properties of the crystal protein. Such a coding sequence will usually be. . .

DETD(164) Accordingly, the polypeptide coded for by the "chimeric" gene of the present invention is preferably structurally related to the delta-"endotoxin" of the crystal protein produced by Bt. Bt produces a crystal protein

with a subunit which is a protoxin having. . . by proteases or by alkali to form insecticidal fragments having an Mr as low as 50,000, and possibly even lower. "Chimeric" genes that code for such fragments of the protoxin or for even smaller portions thereof according to the present invention. . . have the requisite insecticidal activity. The protoxin, insecticidal fragments of the protoxin and insecticidal portions of these fragments can be "fused" to other molecules such as polypeptides.

DETD(189) In addition to the "chimeric" gene coding for a Bt "toxin" or a Bt-like "toxin", the vectors preferably further comprise a DNA sequence that permits the selection or screening of corn plant cells containing the. . . do not contain the vector. Such selectabie or screenable markers may naturally be present in the vector into which the "chimeric" gene of this invention is introduced, or may be introduced into the vector either before or after the "chimeric" gene is introduced. Alternatively, the selectable or screenable marker gene or a portion thereof may first be joined to the desired "chimeric" gene or any portion thereof and the recombined genes or gene segments may be introduced as a unit into the vector. The selectable or screenable marker may itself be "chimeric".

DETD(193) The present invention also includes fertile corn plants, the cells of which contain the "chimeric" gene that expresses a Bt crystal "toxin" or a polypeptide having substantially the insect toxicity properties of Bt crystal "toxin".

DETD(201) The . . . larvae comprising feeding the larvae an insecticidal amount of transgenic Zea mays cells containing a gene coding for a Bacillus "thuringiensis" crystal "toxin" or a polypeptide having substantially the insect toxicity properties of a Bacillus "thuringiensis" crystal protein.

DETD(202) The . . . killing or controlling Lepidopteran larvae comprising feeding the larvae an insecticidal amount of transgenic corn plant cells that contain the "chimeric" gene of the invention. The plant cells may be cultured plant cells, or may be components of living plants. Furthermore,. . . present invention also includes a method for killing Coleopteran larvae by feeding them an insecticidal amount of cells containing the "chimeric" gene having the coding sequence of the Bt var. tenebrionis crystal "toxin" or insecticidal parts thereof.

DETD(252) Example 6a: Construction of pTOX, containing a "chimeric" gene encoding the insecticidal "toxin" gene of Bacillus "thuringiensis" var tenebrionis

DETD(253) A gene encoding the insecticidal crystal protein gene of Bacillus "thuringiensis" var. tenebrionis has been characterized and sequenced [Sekar, V. et al., Proc. Natl. Acad Sci USA, 84 (1987) 7036-7040]. This. . . vector, such as the plasmid pCIB770 [Rothstein, S. et al., Gene, 53 (1987) 153-161]. The plasmid pCIB 770 contains a "chimeric" kanamycin gene for expression in plants, as well as the promoter and terminator of the 35S RNA transcript of CaMV [cauliflower mosaic virus] separated by a unique BamH1 site. The restriction fragment bearing the "toxin" coding sequence is made compatible to the unique BamHI site of pCIB770 by use of the appropriate molecular adapter and. . .

DETD(254) Example 6b: Construction of pSAN, containing a "chimeric" gene encoding the insecticidal "toxin" gene of Bacillus "thuringiensis" strain san diego

DETD(255) A gene encoding the insecticidal protein of Bacillus "thuringiensis" strain san diego has been characterized and sequenced by Hernstadt et al., EP-0-202-739 and EP-0-213-818. This coding sequence is isolated. . . convenient restriction fragment and inserted into the appropriate plant expression vector, such as pCIB 770. The plasmid pCIB770 contains a "chimeric" kanamycin gene for expression in plants, as well as the promoter and terminator of the 35S RNA transcript of CaMV [cauliflower mosaic virus] separated by a unique BamH site. The restriction fragment bearing the "toxin" coding sequence is made compatible to the unique BamHII site of PCIB 770 by use of the appropriate molecular adapter. . .

CLMS(1) What . . . transgenic Zea mays cells containing a synthetic DNA which encodes a polypeptide having the insect toxicity properties of a Bacillus "thuringiensis" crystal protein wherein the cells have been grown or cultured in a manner to permit expression of the "toxin" in the cells, wherein said cells comprise a part of a fertile Zea mays plant, wherein said insect larvae is. . .

CLMS(4) 4. The method according to claim 1, wherein said synthetic DNA is a "chimeric" gene.

CLMS(5) 5. The method according to claim 1, wherein said synthetic DNA encodes a Bacillus "thuringiensis" crystal protein.

CLMS(6) 6. . . . transgenic Zea mays cells containing an isolated DNA which encodes a polypeptide having the insect toxicity properties of a Bacillus "thuringiensis" crystal protein wherein the cells have been grown or cultured in a manner to permit expression of the "toxin" in the cells, wherein said cells comprise a part of a fertile Zea mays plant, wherein said insect larvae is. . .

CLMS(9) 9. The method according to claim 6, wherein said isolated DNA is a "chimeric" gene.

CLMS(10) 10. The method according to claim 6, wherein said isolated DNA encodes a Bacillus "thuringiensis" crystal protein.

CLMS(11) 11. . . . transgenic Zea mays cells containing an isolated DNA which encodes a polypeptide having the insect toxicity properties of a Bacillus "thuringiensis" crystal protein wherein the cells have been grown or cultured in a manner to permit expression of the "toxin" in the cells, wherein said insect larvae is selected from the group consisting of lepidopteran, coleopteran and dipteran insect larvae.

CLMS(14) 14. The method according to claim 11, wherein said isolated DNA is a "chimeric" gene.

CLMS(15) 15. The method according to claim 11, wherein said isolated DNA encodes a Bacillus "thuringiensis" crystal protein.

CLMS(16) 16. . . . transgenic Zea mays cells containing a synthetic DNA which encodes a polypeptide having the insect toxicity properties of a Bacillus "thuringiensis" crystal protein wherein the cells have been grown or cultured in a manner to permit expression of the "toxin" in the cells, wherein said insect larvae is selected from the group consisting of lepidopteran, coleopteran and dipteran insect larvae.

CLMS(19) 19. The method according to claim 16, wherein said synthetic DNA is a "chimeric" gene.

CLMS(20) 20. The method according to claim 16, wherein said synthetic DNA encodes a Bacillus "thuringiensis" crystal protein.

CLMS(21) 21. . . . of transgenic Zea mays cells containing a gene which encodes a polypeptide having the insect toxicity properties of a Bacillus "thuringiensis" crystal protein wherein the cells have been grown or cultured in a manner to permit expression of the "toxin" in the cells, wherein said insect larvae is selected from the group consisting of lepidopteran, coleopteran and dipteran insect larvae.

CLMS(23) 23. The method according to claim 21, wherein said gene is a "chimeric" gene.

CLMS(24) 24. The method according to claim 21, wherein said gene encodes a Bacillus "thuringiensis" crystal protein.

CLMS(27) 27. The method according to claim 25, wherein said gene is a "chimeric" gene.

CLMS(28) 28. The method according to claim 25, wherein said gene encodes a Bacillus "thuringiensis" crystal protein.

US PAT NO: 5,770,450 [IMAGE AVAILABLE] L10: 7 of 31

ABSTRACT: Methods . . . derived from embryogenic cell cultures or callus cultures. The protoplasts, cells and resulting plants may be transgenic, containing, for example, "chimeric" genes coding for a polypeptide having substantially the insect toxicity properties of the crystal protein produced by Bacillus "thuringiensis".

BSUM(12) Bacillus "thuringiensis" (hereinafter Bt) is a species of bacteria that produces a crystal protein, also referred to as delta-"endotoxin". This crystal protein is, technically, a protoxin that is converted into a "toxin" upon being ingested by larvae of lepidopteran, coleopteran and dipteran insects.

BSUM(32) This invention further provides "chimeric" genes capable of expressing in corn cells a polypeptide having substantially the insect toxicity properties of BT crystal protein (hereinafter, "chimeric" BT "toxin" gene).

BSUM(33) Additional embodiments of the present invention include the "chimeric" BT "toxin" gene in vectors, bacteria, plant cells in culture, and plant cells in living plants, as well as methods for producing a "toxin" having substantially the insect toxicity properties of BT crystal protein in corn cells and methods for controlling or killing insects by feeding them corn cells containing a gene that expresses such a "toxin".

BSUM(51) It . . . provide a method of controlling insect larvae, preferably lepidopteran, coleopteran and dipteran larvae by feeding them corn plant cells containing "chimeric" genes which express an insecticidal amount of a BT crystal "toxin" or a "toxin" having substantially the insect toxicity properties of Bt.beta..beta. crystal protein

DRWDD(23) FIGS. 13A-13E show the nucleotide sequence of the "endotoxin" gene from Bacillus "thuringiensis" var. kurstaki HD1. A preferred sequence of nucleotides that codes for a crystal protein is that shown as nucleotides 156. . .

DETD(140) The present invention is directed to the production of a "chimeric" Bt "toxin" gene. The corn plant cells contemplated include cells from all genotypes (varieties, cultivars, inbred lines, hybrids, etc.) of corn plants. . .

DETD(158) The coding region of the "chimeric" gene contains a nucleotide sequence that codes for a polypeptide having substantially the toxicity properties of a Bt delta-"endotoxin" crystal protein. A polypeptide, for the purpose of the present invention, has substantially the toxicity properties of Bt delta-"endotoxin" crystal protein if it is insecticidal to a similar range of insect larvae as is the crystal protein from a. . .

DETD(160) The coding sequence of the "chimeric" gene may also code for a polypeptide that differs from a naturally occurring crystal protein delta-"endotoxin" but that still has substantially the insect toxicity properties of the crystal protein. Such a coding sequence will usually be. . .

DETD(163) Accordingly, the polypeptide coded for by the "chimeric" gene of the present invention is preferably structurally related to the delta-"endotoxin" of the crystal protein produced by Bt. Bt produces a crystal protein with a subunit which is a protoxin having. . . by proteases or by alkali to form insecticidal fragments having an Mr as low as 50,000, and possibly even lower. "Chimeric" genes that code for such fragments of the protoxin or for even smaller portions thereof according to the present invention. . . have the requisite insecticidal activity. The protoxin, insecticidal fragments of the protoxin and insecticidal portions of these fragments can be "fused" to other molecules such as polypeptides.

DETD(187) In addition to the "chimeric" gene coding for a Bt "toxin" or a Bt-like "toxin", the vectors preferably further comprise a DNA sequence that permits the selection or screening of corn plant cells containing the. . . do not contain the vector. Such selectable or screenable markers may naturally be present in the vector into which the "chimeric" gene of this invention is introduced, or may be introduced into the vector either before or after the "chimeric" gene is introduced. Alternatively, the selectable or screenable marker gene or a portion thereof may first be joined to the desired "chimeric" gene or any portion thereof and the recombined genes or gene segments may be introduced as a unit into the vector. The selectable or screenable marker may itself be "chimeric".

DETD(191) The present invention also includes fertile corn plants, the cells of which contain the "chimeric" gene that expresses a Bt crystal "toxin" or a polypeptide having substantially the insect toxicity properties of Bt crystal "toxin".

DETD(199) The . . . larvae comprising feeding the larvae an insecticidal amount of transgenic Zea mays cells containing a gene coding for a Bacillus "thuringiensis" crystal "toxin" or a polypeptide having substantially the insect toxicity properties of a Bacillus "thuringiensis" crystal protein.

DETD(200) The . . . killing or controlling Lepidopteran larvae comprising feeding the larvae an insecticidal amount of transgenic corn plant cells that contain the "chimeric" gene of the invention. The plant cells may be cultured plant cells, or may be components of living plants. Furthermore,. . . present invention also includes a method for killing Coleopteran larvae by feeding them an insecticidal amount of cells containing the "chimeric" gene having the coding sequence of the Bt var. tenebrionis crystal "toxin" or insecticidal parts thereof.

DETD(250) Example 6a: Construction of pTOX, containing a "chimeric" gene encoding the insecticidal "toxin" gene of Bacillus "thuringiensis" var tenebrionis

DETD(251) A gene encoding the insecticidal crystal protein gene of Bacillus "thuringiensis" var. tenebrionis has been characterized and sequenced [Sekar, V. et al., Proc. Natl. Acad Sci USA, 84 (1987) 7036-7040]. This. . . such as the plasmid pCIB 770 [Rothstein, S. et al., Gene, 53 (1987) 153-161]. The plasmid pCIB 770 contains a "chimeric" kanamycin gene for expression in plants, as well as the promoter and terminator of the 35S RNA transcript of CaMV [cauliflower mosaic virus] separated by a unique BamHI site. The restriction fragment bearing the "toxin" coding sequence is made compatible to the unique BamHI site of pCIB 770 by use of the appropriate molecular adapter. . .

DETD(252) Example 6b: Construction of PSAN, containing a "chimeric" gene encoding the insecticidal "toxin" gene of Bacillus "thuringiensis" strain san diego

DETD(253) A gene encoding the insecticidal protein of Bacillus "thuringiensis" strain san diego has been characterized and sequenced by Hernstadt et al., EP-0-202-739 and EP-0-213-818. This coding sequence is isolated. . . convenient restriction fragment and inserted into the appropriate plant expression vector, such as pCIB 770. The plasmid pCIB770 contains a "chimeric" kanamycin gene for expression in plants, as well as the promoter and terminator of the 35S RNA transcript of CaMV [cauliflower mosaic virus] separated by a uniq[redacted] BamH site. The restriction fragment bearing the "toxin" coding sequence is made compatible to the unique BamHI site of pCIB 770 by use of the appropriate molecular adapter. .

. US PAT NO: 5,767,372 [IMAGE AVAILABLE] L10: 8 of 31

TITLE: Transformation vectors allowing expression of foreign polypeptide endotoxins from Bacillus "thuringiensis" in plants

ABSTRACT: Novel transformation vectors containing novel "chimeric" genes allow the introduction of exogenous DNA fragments coding for polypeptide toxins produced by Bacillus "thuringiensis" or having substantial sequence homology to a gene coding for a polypeptide "toxin" as described herein and expression of the "chimeric" gene in plant cells and their progeny after integration into the plant cell genome. Transformed plant cells and their progeny exhibit stably inherited polypeptide "toxin" expression useful for protecting said plant cells and their progeny against certain insect pests and in controlling said insect pests.

BSUM(3) This . . . the use of genetic engineering techniques in the modification of plants. More particularly, it concerns introduction and integration of a "chimeric" gene coding for a polypeptide "toxin" produced by Bacillus "thuringiensis" or having substantial sequence homology to a "toxin" gene described below in plant cells and obtaining an insect controlling level of expression of said polypeptide "toxin" intra-cellulary by transformed plant cells and their progeny.

BSUM(8) Bacillus "thuringiensis" (referred to at times herein as B.t.) bacteria includes approximately 19 known varieties that produce polypeptide toxins which form parasporal. . . by insect larvae, the crystals are solubilized and processed in the insect midgut to yield at least one active polypeptide "toxin" which is believed to act on the midgut cell membrane. Studies have revealed that individual crystal polypeptides exhibit insecticidal activity.. . .

BSUM(13) It is one object of this invention to provide novel "chimeric" genes coding for the polypeptide "toxin" produced by Bacillus "thuringiensis", or coding for a polypeptide "toxin" having substantial sequence homology to a "toxin" gene described herein. The "chimeric" genes' plant regulatory sequences direct expression in transformed plant cells.

BSUM(20) (b) at least one DNA fragment coding for a polypeptide "toxin" produced by Bacillus "thuringie[redacted] having substantial sequence homology thereto.

BSUM(27) (ii) at least one DNA fragment coding for a polypeptide "toxin" produced by Bacillus "thuringiensis" or at least one DNA fragment having substantial sequence homology thereto.

BSUM(31) (b) at least one DNA fragment coding for a polypeptide "toxin" produced by Bacillus "thuringiensis", or at least one DNA fragment having substantial sequence homology thereto.

BSUM(35) Transformed plant cells and their progeny intracellulary express a polypeptide "toxin" substantially similar to the polypeptide toxins produced by Bacillus "thuringiensis" and are substantially toxic to certain insects. Transformed plant cells and their progeny may be used in controlling said insects.

DETD(7) (1) isolation of at least one DNA fragment from Bacillus "thuringiensis" coding for a polypeptide "toxin" by digestion of bacterial DNA and inserting the mixture or DNA fragments obtained into a cloning vehicle harbored in a. . .

DETD(25) Transformed plant cells and their progeny should express a polypeptide "toxin" substantially similar to polypeptide toxins being produced by Bacillus "thuringiensis" or a DNA fragment having substantial sequence homology to Bt2.

DETD(68) Straight promotor-gene "fusions" in which only part of the Bt2 coding sequence is used ("truncated Bt2"). Fragments of the Bt2 sequence still encoding an active "toxin" are inserted behind the plant specific promoters: The toxic polypeptides produced in the plant cells using these constructs should have. . .

DETD(71) Straight promotor-gene "fusions" in which a Bt:NPTII "fusion" gene (also referred to at times at Bt2:NPTII) is inserted behind the promotor. "Fusion" genes were constructed, consisting of a fragment of the Bt2 coding sequence (still encoding an active "toxin") "fused" to the coding sequence of the NPTII enzyme. The Bt:NPTII "fusion" genes used here, specify stable "fusion" proteins comprising amino terminal parts of the Bt2 protein "fused" to an intact Neomycin phosphotransferase (NTPII) enzyme. These "fusion" proteins have a specific toxicity comparable to the intact Bt2 protein and retain neomycin phosphotransferase enzyme activity. Thus, expression of the Bt:NPTII "fusion" proteins in plant cells allows direct selection for the production of this protein by isolating Kanamycin resistant (Km.sup.R) transformed cells. . . to a high level of Kanamycin should identify, among all possible transformations, those which produce high levels of the toxic "fusion" protein. Further, expression of the "fusion" protein by a Bt:NPTII "fusion" gene might have other desirable properties such as stability in plant cells; for example, mRNA may be more stable. Differences in results obtained with these Type IV "fusion" genes might be due to intrinsic differences in the properties of the "fusion" protein expressed as compared to the intact Bt2 protein.

DETD(88) Kronstad et al., J. Bacteriol., 54, p. 419-428 (1983) reported that B.t. berliner 1715 contains two related "toxin" genes which are both located on plasmids. Intact "endotoxin" genes were isolated from a gene bank from total B.t. berliner 1715 plasmid DNA using partial Sau3A digests of plasmid. . . DNA. The pEcoR251 plasmid is a derivative of plasmid pBR322 in which the EcoRI-PuvII fragment has been replaced by a "chimeric" EcoRI endonuclease gene which is "fused" to a PR promotor fragment derived from plasmid pLK5 (Zabeau and Stanley, EMBO Journal, 1, 1217-1224 (1982)) as depicted in. . .

DETD(135) The previous data suggests that the smallest gene fragment of Bt2, encoding an active "toxin" is contained within the KpnI deletion fragment but extends further than the HindIII site. To map the exact endpoint of the minimal fragment coding for the active "toxin", deletion mutants were constructed which contained N-terminal fragments or decreasing size. To achieve this, we used a strategy which allowed us to construct simultaneously deletion-mutants and translational "fusions" to the NPTII-gene (see Section 7.2.2). The construction of the intermediate plasmid pLBKm25 is outlined in FIG. 18. As shown. . .

DETD(136) As . . . Bal31, cut with SalI, treated with Klenow polymerase and relegated (FIG. 19). In this way, the deleted coding region is "fused" to a stopcodon with a minimum of nonsense coding sequence. An overview of the deletion clones is given in FIG.. . . blotting and ELISA for the quantitative detection of Bt2-like polypeptides and in an insect toxicity assay to screen for active "toxin". The results are presented in FIG. 21 and indicate that detection of a stable polypeptide decreases gradually when the endpoint. . .

DETD(141) Since NPTII is a most suitable selection marker in plant engineering, such gene "fusions" could have very promising applications. Indeed when using such NPTII "fusion" proteins to transform plants, a selection for high kanamycin resistance would allow direct selection for a high expression of the "fusion" product. Therefore, "toxin" gene "fusions" with NPTII might be used to transform plants and select for transformed plants expressing high levels of "toxin", by selection for kanamycin resistance.

DETD(170) Previous . . . on the identification of minimal active toxic fragments have shown that this Kpn fragment comprises a (approximately 60 Kd) active "toxin" which exhibits the complete toxic activity or the Bt2 molecule. In the following, we wanted to determine whether the Bt:NPT2 "fusion" protein had still the same degree of toxicity.

DETD(176) 145 . . . concentrations. 8 transformants proved more resistant and were able to grow on concentrations higher than 200 ug/ml of kanamycin. The "fusion" point in all 8 clones was determined by restriction enzyme mapping with an accuracy of 20 bp. Surprisingly 7 out of 8 clones had their "fusion" point around the HindIII site at position 1680 of the Bt gene. One clone (pLBKm860) mapped at position approximately 2050. Although the majority of the deletions were "fused" around position 1800, none of these conferred a higher kanamycin resistant phenotype. The 7 clones which have their "fusion" point positioned around the HindIII site are too short to encode an active "toxin". However, one of the clones (pLBKm860) was:

DETD(186) Table . . . is the result of a cointegration of a receptor Ti plasmid with an intermediate vector. Each intermediate vector contains a "chimeric" "toxin" gene comprising a plant promotor sequence derived from the indicated expression vector and a Bt gene cassette.

DETD(221) This example describes the construction of pHD205, an intermediate vector containing a "chimeric" Bt2 "toxin" gene comprising: the nopaline synthase promotor, the Bt2 "toxin" gene cassette from pHD160 and a DNA fragment containing the 3' untranslated region of the nopaline synthase gene including the polyadenylation site. In the "chimeric" gene the Bt2 gene cassette is oriented such that the expression of the Bt2 protein can be obtained from the. . . are fragments of approximately 6200 bp, 3000 bp, 1800 bp and 920 bp. A recombinant plasmid with the alpha-orientation (the "toxin" gene under the control of the nopaline synthase promotor) is used in subsequent experiments and called pHD205.

DETD(223) This example describes the construction of pHD208. The intermediate vector pHD208 contains a "chimeric" Bt2 "toxin" gene comprising: the promotor from a pea gene encoding a small subunit of ribulose biphosphate carboxylase (Pssu), the Bt2 "toxin" gene cassette from pHD160 and the 3' untranslated region of the octopine synthase gene including the polyadenylation site. The fragments of the "chimeric" gene were assembled in the cloning vector pGV831 as described in this example and as diagrammed in FIG. 29. The. . .

DETD(267) 10. Isolation of plant cells and plants containing the "chimeric" "toxin" gene inserted in their genome

DETD(479) A . . . transformation vectors described herein will contain, stably inserted into their genome, a fragment of newly acquired DNA containing both a "chimeric" Bt "toxin" gene and a marker gene (nos, NPTII). This was confirmed by the results of southern blotting experiments. The new phenotypic traits acquired through this transformation method (expression of Bt "Toxin", antibiotic resistance, nopaline production) will be inherited according to classic Mendelian genetics. To verify stable inheritance of the new traits, F.sub.1 descendants from transformed plants were analysed for the expression of Bt "toxin" and synthesis of nopaline.

DETD(492) TABLE 4

T
oxicity of Bt:NPT2 "Fusion" Protein on 3rd Instar P. brassicae (% Mortality After 4 Days)

"Toxin" dose (ug/ml) Bt protein	0.1	0.2	0.3	0.6	1
Bt2 70 NT.sup.(x)	90	NT	100	Bt:NPT2	NT. . .

DETD(493) TABLE 5

Toxicity of Intact Bt2 Protein, 60 Kd "Processed" Bt2 Protein (Trypsin Digested) and Bt:NPT2 "Fusion" Protein on Larvae of Manduca Sexta

	% Mortality after 4 days *						
"Toxin" dose: (ng/cm.sup.2)	0	0.67	2	6	18	54	162
130 Kd Bt2	0	0	0	0	3	8. . .	20.7
60 Kd Bt2	--	16.3	8.3	6.4	3.9		
Bt:NPT2	--	26.5	15.8	7.7	4.5		

130 Kd Bt2	0	0	0	0	3	8. . .	20.7	9.4	5.4	2.4
60 Kd Bt2	--	16.3	8.3	6.4	3.9					
Bt:NPT2	--	26.5	15.8	7.7	4.5					

"Toxin" dilutions were applied on artificial diet as described in Section 12. Thirty (30) 1st instar larvae were used per. .

We claim:

1. A "chimeric" gene, comprising: a) a first DNA encoding an about 60-80 kD insecticidal protein fragment of a Bacillus "thuringiensis" insecticidal crystal protein; and b) a promoter region and a 3' non-translated region comprising a polyadenylation signal, said promoter and 3'. . .

CLMS(2) 2. The "chimeric" gene of claim 1, wherein the crystal protein has a toxicity to Lepidopteran insects.

CLMS(3) 3. The "chimeric" gene of claim 1, wherein the insecticial protein fragment is of a crystal protein of Bacillus "thuringiensis" berliner 1715, of Bacillus "thuringiensis" kurstaki or of Bacillus "thuringiensis" sotto.

CLMS(4) 4. The "chimeric" gene of claim 1, wherein the insecticidal protein fragment is of the Bt2 crystal protein with the amino acid sequence. . .

CLMS(5) 5. The "chimeric" gene of claim 1, wherein the promoter region is a tissue-specific or inducible promoter region, or is a promoter region. . .

CLMS(6) 6. The "chimeric" gene of claim 1, wherein the 3' non-translated region is from an octopine synthase gene, a T-DNA gene 7, a. . .

CLMS(7) 7. The "chimeric" gene of claim 1, which further comprises a second DNA encoding an enzyme capable of being expressed in a plant cell and the expression of which can be identified in the cell; the second DNA fragment being "fused" to the first DNA fragment so that the first and second DNAs encode a "fusion" polypeptide.

CLMS(8) 8. The "chimeric" gene of claim 7, wherein the second DNA encodes a selectable or scorable marker.

CLMS(9) 9. The "chimeric" gene of claim 8, wherein the second DNA encodes a neomycin phosphotransferase.

CLMS(10) 10. The "chimeric" gene of claim 1, which further comprises a second DNA encoding a transit peptide upstream of said first DNA encoding said insecticidal protein fragment so that a transit peptide-insecticidal protein fragment "fusion" protein is encoded by said "chimeric" gene.

CLMS(11) 11. The "chimeric" gene of claim 1, wherein said DNA encodes an about 60 kD insecticidal protein fragment that has been truncated near. . .

CLMS(12) 12. The "chimeric" gene of claim 1, wherein said coding region encodes an about 60 kD protein fragment of the Bt2 protein of. . .

CLMS(13) 13. A plant comprising the "chimeric" gene of claim 1, stably inserted into the genome of the plant.

CLMS(14) 14. A "chimeric" gene comprising: (1) a coding region comprising a DNA encoding an about 60 kD active "toxin" of the Bt2 insecticidal crystal protein of SEQ ID No. 1, (2) a promoter region and a 3' non-translated region comprising. . .

CLMS(15) 15. The "chimeric" gene of claim 14, wherein said coding region encodes a "fusion" protein of an insecticidal fragment of about 60 to about 80 kD of the Bt2 protein of SEQ ID No.. . .

CLMS(16) 16. The "chimeric" gene of claim 14, wherein said coding region encodes a "fusion" protein of an insecticidal fragment of about 60 to about 80 kD of the Bt2 protein of SEQ ID No.. . .

CLMS(17) 17. The "chimeric" gene of claim 14 including a translation initiation site comprising the sequence ATGGATCCC wherein the codon ATG in this sequence. .

. US PAT NO: 5,766,900 [IMAGE AVAILABLE] L10: 9 of 31

ABSTRACT: Methods . . . derived from embryogenic cell cultures or callus cultures. The protoplasts, cells and resulting plants may be transgenic, containing, for example, "chimeric" genes coding for a polypeptide having substantially the insect toxicity properties of the crystal protein produced by Bacillus "thuringiensis".

BSUM(12) Bacillus "thuringiensis" (hereinafter Bt) is a species of bacteria that produces a crystal protein, also referred to as delta-"endotoxin". This crystal protein is, technically, a protoxin that is converted into a "toxin" upon being ingested by larvae of lepidopteran, coleopteran and dipteran insects.

BSUM(32) This invention further provides "chimeric" genes capable of expressing in corn cells a polypeptide having substantially the insect toxicity properties of Bt crystal protein (hereinafter, "chimeric" Bt "toxin" gene).

BSUM(33) Additional embodiments of the present invention include the "chimeric" Bt "toxin" gene in vectors, bacteria, plant cells in culture, and plant cells in living plants, as well as methods for producing a "toxin" having substantially the insect toxicity properties of Bt crystal protein in corn cells and methods for controlling or killing insects by feeding them corn cells containing a gene that expresses such a "toxin".

BSUM(51) It . . . provide a method of controlling insect larvae, preferably lepidopteran, coleopteran and dipteran larvae by feeding them corn plant cells containing "chimeric" genes which express an insecticidal amount of a Bt crystal "toxin" or a "toxin" having substantially the insect toxicity properties of Bt .alpha..beta. crystal protein

DRWDD(25) FIG. 13 shows the nucleotide sequence of the "endotoxin" gene from Bacillus "thuringiensis" var. kurstaki HD1. A preferred sequence of nucleotides that codes for a crystal protein is that shown as nucleotides 156. . .

DETD(140) The present invention is directed to the production of a "chimeric" Bt "toxin" gene. The corn plant cells contemplated include cells from all genotypes (varieties, cultivars, inbred lines, hybrids, etc.) of corn plants. . .

DETD(158) The coding region of the "chimeric" gene contains a nucleotide sequence that codes for a polypeptide having substantially the toxicity properties of a Bt delta-"endotoxin" crystal protein. A polypeptide, for the purpose of the present invention, has substantially the toxicity properties of Bt delta-"endotoxin" crystal protein if it is insecticidal to a similar range of insect larvae as is the crystal protein from a. . .

DETD(160) The coding sequence of the "chimeric" gene may also code for a polypeptide that differs from a naturally occurring crystal protein delta-"endotoxin" but that still has substantially the insect toxicity properties of the crystal protein. Such a coding sequence will usually be. . .

DETD(163) Accordingly, the polypeptide coded for by the "chimeric" gene of the present invention is preferably structurally related to the delta-"endotoxin" of the crystal protein produced by Bt. Bt produces a crystal protein with a subunit which is a protoxin having. . . by proteases or by alkali to form insecticidal fragments having an Mr as low as 50,000, and possibly even lower. "Chimeric" genes that code for such fragments of the protoxin or for even smaller portions thereof according to the present invention. . . have the requisite insecticidal activity. The protoxin, insecticidal fragments of the protoxin and insecticidal portions of these fragments can be "fused" to other molecules such as polypeptides

DETD(187) In addition to the "chimeric" gene coding for a Bt "toxin" or a Bt-like "toxin", the vectors preferably further comprise a DNA sequence that permits the selection or screening of corn plant cells containing the. . . do not contain the vector. Such selectable or screenable markers may naturally be present in the vector into which the "chimeric" gene of this invention is introduced, or may be introduced into the vector either before or after the "chimeric" gene is introduced. Alternatively, the selectable or screenable marker gene or a portion thereof may first be joined to the desired "chimeric" gene or any portion thereof and the recombined genes or gene segments may be introduced as a unit into the vector. The selectable or screenable marker may itself be "chimeric".

DETD(191) The present invention also includes fertile corn plants, the cells of which contain the "chimeric" gene that expresses a Bt crystal "toxin" or a polypeptide having substantially the insect toxicity properties of Bt crystal "toxin".

DETD(199) The . . . larvae comprising feeding the larvae an insecticidal amount of transgenic Zea mays cells containing a gene coding for a Bacillus "thuringiensis" crystal "toxin" or a polypeptide having substantially the insect toxicity properties of a Bacillus "thuringiensis" crystal protein.

DETD(200) The . . . killing or controlling Lepidopteran larvae comprising feeding the larvae an insecticidal amount of transgenic corn plant cells that contain the "chimeric" gene of the invention. The plant cells may be cultured plant cells, or may be components of living plants. Furthermore,. . . present invention also includes a method for killing Coleopteran larvae by feeding them an insecticidal amount of cells containing the "chimeric" gene having the coding sequence of the Bt var. tenebrionis crystal "toxin" or insecticidal parts thereof.

DETD(258) Construction of pTOX, Containing a "Chimeric" Gene Encoding the Insecticidal "Toxin" Gene of Bacillus "thuringiensis" var tenebrionis

DETD(259) A gene encoding the insecticidal crystal protein gene of Bacillus "thuringiensis" var. tenebrionis has been characterized and sequenced [Sekar, V. et al., Proc. Natl. Acad Sci USA, 84 (1987) 7036-7040]. This. . . such as the plasmid pCIB 770 [Rothstein, S. et al., Gene, 53 (1987) 153-161]. The plasmid pCIB 770 contains a "chimeric" kanamycin gene for expression in plants, as well as the promoter and terminator of the 35S RNA transcript of CaMV [cauliflower mosaic virus] separated by a unique BamHI site. The restriction fragment bearing the "toxin" coding sequence is made compatible to the unique BamHI site of pCIB 770 by use of the appropriate molecular adapter. . .

DETD(261) Construction of pSAN, Containing a "Chimeric" Gene Encoding the Insecticidal "Toxin" Gene of Bacillus "thuringiensis" Strain San Diego

DETD(262) A gene encoding the insecticidal protein of Bacillus "thuringiensis" strain san diego has been characterized and sequenced by Herrnstadt et al., EP-0-202-739 and EP-0-213-818. This coding sequence is isolated. . . convenient restriction fragment and inserted into the appropriate plant expression vector, such as pCIB 770. The plasmid pCIB770 contains a "chimeric" kanamycin gene for expression in plants, as well as the promoter and terminator of the 35S RNA transcript of CaMV [cauliflower mosaic virus] separated by a unique BamH site. The restriction fragment bearing the "toxin" coding sequence is made compatible to the unique BamHI site of pCIB 770 by use of the appropriate molecular adapter. .

. US PAT NO: 5,763,241 [IMAGE AVAILABLE] L10: 10 of 31

ABSTRACT: A method for producing genetically transformed plants exhibiting toxicity to Coleopteran insects is disclosed. In another aspect, the present invention embraces "chimeric" plant genes, genetically transformed cells and differentiated plants which exhibit toxicity to Coleopteran insects. In yet another aspect, the present invention embraces bacterial cells and plant transformation vectors comprising a "chimeric" plant gene encoding a Coleopteran "toxin" protein of Bacillus "thuringiensis".

BSUM(3) Bacillus "thuringiensis" (B.t.) is a spore forming soil bacterium which is known for its ability to produce a parasporal crystal protein which. . . butterflies) and a few are reported to have activity against Dipteran insects (mosquitoes and flies, see Aronson et al. 1986). "Toxin" genes from a variety of these strains have been cloned and the toxins have been expressed in heterologous hosts (Schnepf. . . var. san diego (B.t. sd., Herrnstadt et al., 1986) strains have been identified as having activity against Coleopteran insects. The "toxin" gene from B.t.sd. has been cloned, but the "toxin" produced in E. coli was reported to be a larger size than the "toxin" from B.t.sd. crystals, and activity of this recorabinant B.t.sd. "toxin" was implied to be weak.

BSUM(4) Insects susceptible to the action of the protein "toxin" of Coleopteran-type Bacillus "thuringiensis" bacteria include, but are not limited to, Colorado potato beetle (Leptinotarsa decemlineata), boll weevil (Anthonomus grandis), yellow mealworm (Tenebrio molitor),. . .

BSUM(5) Although certain "chimeric" genes have been expressed in transformed plant cells and plants, such expression is by no means straight forward. Specifically, the expression of Lepidopteran-type B.t. "toxin" proteins has been particularly problematic. It has now been found that the teachings of the art with respect to expression of Lepidopteran-type B.t. "toxin" protein in plants do not extend to Coleopteran-type B.t. "toxin" protein. These findings are directly contrary to the prior teachings which suggested that one would employ the same genetic manipulations. . .

BSUM(11) ii) a DNA sequence that causes the production of a RNA sequence encoding a Coleopteran-type "toxin" protein of Bacillus "thuringiensis"; and

BSUM(17) (b) a DNA sequence that causes the production of a RNA sequence encoding a Coleopteran-type "toxin" protein of Bacillus "thuringiensis"; and

DETD(2) The . . . plants to exhibit toxicity toward susceptible Coleopteran insects. More particularly, the present invention provides transgenic plants which express the Coleopteran-type "toxin" protein of Bacillus "thuringiensis" at an insecticidal level.

DETD(8) The "chimeric" gene also contains a structural coding sequence which encodes the Coleopteran-type "toxin" protein of Bacillus "thuringiensis" or an insecticidally-active Fragment thereof. Exemplary sources of such structural coding sequences are B.t. tenebrionis and B.t. san diego. Accordingly, in exemplary embodiments the present invention provides a structural coding sequence from Bacillus "thuringiensis" var. tenebrionis and insecticidally-active fragments thereof. Those skilled in the art will recognize that other structural coding sequence substantially homologous to the "toxin" coding sequence of B.t.t. can be utilized following the teachings described herein and are, therefore, within the scope of this. . .

DETD(11) The plant material thus modified can be assayed, for example, by Northern blotting, for the presence of Coleopteran-type "toxin" protein mRNA. If no "toxin" protein mRNA (or too low a titer) is detected, the promoter used in the "chimeric" gene construct is replaced with another, potentially stronger promoter and the altered construct retested. Alternately, level of "toxin" protein may be assayed by immunoassay such as Western blot. In many cases the most sensitive assay for "toxin" protein is insect bioassay.

DETD(25) Using . . . sequence information, synthetic DNA probes (FIG. 1) were designed which were used in the isolation of clones containing the B.t.t. "toxin" gene. Probes were endlabeled with [.lambda.-.sup.32 P] ATP according to Maniatis (1982). B. "thuringiensis" var. tenebrionis was grown for 6 hours at 37.degree. C. in Spizizen medium (Spizizen, 1958) supplemented with 0.1% yeast extract. . .

DETD(60) Although the Coleopteran-type toxins and the Lepidopteran-type toxins are derived from Bacillus "thuringiensis", there are significant differences between the "toxin" genes and the "toxin" proteins of the two types. As isolated from Bacillus "thuringiensis" both types of toxins are found in parasporal crystals; however, as described above, the solubility properties of the crystals are distinctly different. In addition, the sizes of the "toxin" proteins found in solubilized crystals are completely different. Lepidopteran-type "toxin" proteins are typically on the order of 130 kDa while the Coleopteran-type "toxin" proteins are approximately 70 kDa.

DETD(145) "Chimeric" B.t.t. "Toxin" Gene Using a Mas Promoter

DETD(149) "Chimeric" B.t.t. "toxin" genes driven by the MAS promoter are prepared by digesting either pMON9791 or pMON9792 with BglII, recovering the "toxin" encoding fragment and moving this fragment into pMON9741 following the teachings provided herein.

DETD(161) Shoot . . . streaked on an LB agar plate and grown for 2 to 3 days. pMON9753-ASE which is described above contains the "chimeric" B.t.t. "toxin" gene driven by the CaMV35S promoter. Alternatively, Agrobacterium strains pMON9791-ACO or pMON9792-ACO containing "chimeric" B.t.t. "toxin" genes are used. Stem sections are placed on 0.8% agar-solidified medium containing salts and organic addenda as in Jarret et. . . potato cells are transformed. Uninoculated control tissue is inhibited at this concentration of kanamycin. Transformed potato tissue expresses the B.t.t. "toxin" gene. B.t.t. "toxin" mRNA may be detected by Northern analysis and B.t.t. "toxin" protein may be detected by immunoassay such as Western blot analysis. However, in many cases the most sensitive assay for the presence of B.t.t. "toxin" is the insect bioassay. Colorado potato beetle larvae feeding on the transformed tissue suffer from the effects of the "toxin".

DETD(167) When the Agrobacterium strain used for transformation contains a "chimeric" B.t.t. "toxin" gene such as pMON9753, pMON9791 or pMON9792, the B.t.t. "toxin" gene is expressed in the transformed callus, embryos derived from this callus, and in the transformed plants derived from the embryos. For all of these cases, expression of the B.t.t. "toxin" mRNA may be detected by Northern analysis, and expression of the B.t.t. "toxin" protein may be detected by immunoassay such as Western blot analysis. Insect bioassay may be the most sensitive measure for the presence of "toxin" protein.

DETD(170) The following description outlines the preparation of protoplasts from maize, the introduction of "chimeric" B.t.t. "toxin" genes into the protoplast by electroporation, and the recovery of stably transformed, kanamycin resistant maize cells expressing "chimeric" B.t.t. "toxin" genes.

DETD(176) As . . . al. (1986), transformed maize cells can be selected by growth in kanamycin containing medium following electroporation with DNA vectors containing "chimeric" kanamycin resistance genes composed of the CaMV35S promoter, the NPTII coding region and the NOS 3' end. pMON9791 and pMON9792 contain such "chimeric" NPTII genes and also contain "chimeric" B.t.t. "toxin" genes. As described above, maize protoplasts are transformed by electroporation with DNA vectors where the DNA vectors are pMON9791 or pMON9792. Following selection for kanamycin resistance, the transformed maize cells are assayed for expression of the B.t.t. "toxin" gene. Assays are performed for B.t.t. mRNA by Northern blot analysis and for B.t.t. "toxin" protein by immunoassay such as Western blot analysis.

CLMS(1) We . . . exhibits toxicity toward Coleopteran insects which comprises the steps of: (a) inserting into the genome of a plant cell a "chimeric" gene which comprises in sequence: i) a promoter which functions in plants to cause the production of RNA; ii) a DNA sequence that causes the production of a RNA sequence encoding Coleopterantype "toxin" protein of Bacillus "thuringiensis" var. tenebrionis having the amino acid sequence selected from the group consisting of from residues (1-644), residues (16-644), residues (48-644),. . .

CLMS(9) 9. The method of claim 1 in which said DNA sequence encodes the "toxin" protein of Bacillus "thuringiensis" var. tenebrionis having the amino acid sequence from residues (1-644) of said protein wherein the amino acid residues of said. . .

CLMS(10) 10. The method of claim 1 in which said DNA sequence encodes the "toxin" protein of Bacillus "thuringiensis" var. tenebrionis having the amino acid sequence from residues (16-644) of said protein wherein the amino acid residues of said. . .

CLMS(11) 11. . . . exhibits toxicity toward Coleopteran insects which comprises the steps of: (a) inserting into the genome of a plant cell a "chimeric" gene which comprises in sequence: i) a promoter which functions in plants to cause the production of RNA; ii) a DNA sequence that causes the production of a RNA sequence encoding Coleopterantype "toxin" protein of Bacillus "thuringiensis" var. tenebrionis having the amino acid sequence from residues (48-644) of said protein wherein the amino acid residues of said. . .

CLMS(12) 12. The method of claim 1 in which said DNA sequence encodes the "toxin" protein of Bacillus "thuringiensis" var. tenebrionis having the amino acid sequence from residues (50-644) of said protein wherein the amino acid residues of said. . .

CLMS(13) 13. The method of claim 1 in which said DNA sequence encodes the "toxin" protein of Bacillus "thuringiensis" var. tenebrionis having the amino acid sequence from residues (58-644) of said protein wherein the amino acid residues of said. . .

CLMS(14) 14. The method of claim 1 in which said DNA sequence encodes the "toxin" protein of Bacillus "thuringiensis" var. tenebrionis having the amino acid sequence from residues (77-644) of said protein wherein the amino acid residues of said. .

. US PAT NO: 5,760,181 [IMAGE AVAILABLE] L10: 11 of 31

ABSTRACT: Novel transformation vectors containing novel "chimeric" genes allow the introduction of exogenous DNA fragments coding for polypeptide toxins produced by Bacillus "thuringiensis" or having substantial sequence homology to a gene coding for a polypeptide "toxin" as described herein and expression of the "chimeric" gene in plant cells and their progeny after integration into the plant cell genome. Transformed plant cells and their progeny exhibit stably inherited polypeptide "toxin" expression useful for protecting said plant cells and their progeny against certain insect pests and in controlling said insect pests.

BSUM(2) This . . . the use of genetic engineering techniques in the modification of plants. More particularly, it concerns introduction and integration of a "chimeric" gene coding for a polypeptide "toxin" produced by Bacillus "thuringiensis" or having substantial sequence homology to a "toxin" gene described below in plant cells and obtaining an insect controlling level of expression of said polypeptide "toxin" intra-cellularly by transformed plant cells and their progeny.

BSUM(7) Bacillus "thuringiensis" (referred to at times herein as B.t.) bacteria includes approximately 19 known varieties that produce polypeptide toxins which form parasporal. . . by insect larvae, the crystals are solubilized and processed in the insect midgut to yield at least one active polypeptide "toxin" which is believed to act on the midgut cell membrane. Studies have revealed that individual crystal polypeptides exhibit insecticidal activity.. . .

BSUM(12) It is one object of this invention to provide novel "chimeric" genes coding for the polypeptide "toxin" produced by Bacillus "thuringiensis", or coding for a polypeptide "toxin" having substantial sequence homology to a "toxin" gene described herein. The "chimeric" genes' plant regulatory sequences direct expression in transformed plant cells.

BSUM(19) (b) at least one DNA fragment coding for a polypeptide "toxin" produced by Bacillus "thuringiensis" or having substantial sequence homology thereto.

BSUM(26) (ii) at least one DNA fragment coding for a polypeptide "toxin" produced by Bacillus "thuringiensis" or at least one DNA fragment having substantial sequence homology thereto.

BSUM(30) (b) at least one DNA fragment coding for a polypeptide "toxin" produced by Bacillus "thuringiensis", or at least one DNA fragment having substantial sequence homology thereto.

BSUM(34) Transformed plant cells and their progeny cellularly express a polypeptide "toxin" substantially similar to the polypeptide toxins produced by Bacillus "thuringiensis" and are substantially toxic to certain insects. Transformed plant cells and their progeny may be used in controlling said insects.

DETD(7) (1) isolation of at least one DNA fragment from Bacillus "thuringiensis" coding for a polypeptide "toxin" by digestion of bacterial DNA and inserting the mixture of DNA fragments obtained into a cloning vehicle harbored in a. . .

DETD(25) Transformed plant cells and their progeny should express a polypeptide "toxin" substantially similar to polypeptide toxins being produced by Bacillus "thuringiensis" or a DNA fragment having substantial sequence homology to Bt2.

DETD(68) Straight promotor-gene "fusions" in which only part of the Bt2 coding sequence is used ("truncated Bt2"). Fragments of the Bt2 sequence still encoding an active "toxin" are inserted behind the plant specific promoters: The toxic polypeptides produced in the plant cells using these constructs should have. . .

DETD(71) Straight promotor-gene "fusions" in which a Bt:NPTII "fusion" gene (also referred to at times at Bt2:NPTII) is inserted behind the promotor. "Fusion" genes were constructed, consisting of a fragment of the Bt2 coding sequence (still encoding an active "toxin") "fused" to the coding sequence of the NPTII enzyme. The Bt:NPTII "fusion" genes used here, specify stable "fusion" proteins comprising amino terminal parts of the Bt2 protein "fused" to an intact Neomycin phosphotransferase (NTPII) enzyme. These "fusion" proteins have a specific toxicity comparable to the intact Bt2 protein and retain neomycin phosphotransferase enzyme activity. Thus, expression of the Bt:NPTII "fusion" proteins in plant cells allows direct selection for the production of this protein by isolating Kanamycin resistant (Km.sup.R) transformed cells.. . . to a high level of Kanamycin should identify, among all possible transformations, those which produce high levels of the toxic "fusion" protein. Further, expression of the "fusion" protein by a Bt:NPTII "fusion" gene might have other desirable properties such as stability in plant cells; for example, mRNA may be more stable. Differences in results obtained with these Type IV "fusion" genes might be due to intrinsic differences in the properties of the "fusion" protein expressed as compared to the intact Bt2 protein.

DETD(88) Kronstad et al., J. Bacteriol., 54, p. 419-428 (1983) reported that B.t. berliner 1715 contains two related "toxin" genes which are both located on plasmids. Intact "endotoxin" genes were isolated from a gene bank from total B.t. berliner 1715 plasmid DNA using partial Sau3A digests of plasmid. . . DNA. The pEcoR251 plasmid is a derivative of plasmid pBR322 in which the EcoRI-PuvII fragment has been replaced by a "chimeric" EcoRI endonuclease gene which is "fused" to a P.sub.R promotor fragment derived from plasmid pLK5 (Zabeau and Stanley, EMBO Journal, 1, 1217-1224 (1982)) as depicted in. . .

DETD(135) The previous data suggests that the smallest gene fragment of Bt2, encoding an active "toxin" is contained within the KpnI deletion fragment but extends further than the HindIII site. To map the exact endpoint of the minimal fragment coding for the active "toxin", deletion mutants were constructed which contained N-terminal fragments or decreasing size. To achieve this, we used a strategy which allowed us to construct simultaneously deletion-mutants and translational "fusions" to the NPTII-gene (see Section 7.2.2). The construction of the intermediate plasmid pLBKm25 is outlined in FIG. 18. As shown. . .

DETD(136) As . . . Bal31, cut with SalI, treated with Klenow polymerase and relegated (FIG. 19). In this way, the deleted coding region is "fused" to a stopcodon with a minimum of nonsense coding sequence. An overview of the deletion clones is given in FIG.. . . blotting and ELISA for the quantitative detection of Bt2-like polypeptides and in an insect toxicity assay to screen for active "toxin". The results are presented in FIG. 21 and indicate that detection of a stable polypeptide decreases gradually when the endpoint. . .

DETD(141) Since NPTII is a most suitable selection marker in plant engineering, such gene "fusions" could have very promising applications. Indeed when using such NPTII "fusion" proteins to transform plants, a selection for high kanamycin resistance would allow direct selection for a high expression of the "fusion" product. Therefore, "toxin" gene "fusions" with NPTII might be used to transform plants and select for transformed plants expressing high levels of "toxin", by selection for kanamycin resistance.

DETD(170) Previous . . . on the identification of minimal active toxic fragments have shown that this Kpn fragment comprises a (approximately 60 Kd) active "toxin" which exhibits the complete toxic activity of the molecule. In the following, we wanted to determine whether the Bt:NPT2 "fusion" protein had still the same degree of toxicity.

DETD(176) 145 . . . concentrations. 8 transformants proved more resistant and were able to grow on concentrations higher than 200 ug/ml of kanamycin. The "fusion" point in all 8 clones was determined by restriction enzyme mapping with an accuracy of 20 bp. Surprisingly 7 out of 8 clones had their "fusion" point around the HindIII site at position 1680 of the Bt gene. One clone (pLBKm860) mapped at position approximately 2050. Although the majority of the deletions were "fused" around position 1800, none of these conferred a higher kanamycin resistant phenotype. The 7 clones which have their "fusion" point positioned around the HindIII site are too short to encode an active "toxin". However, one of the clones (pLBKm860) was:

DETD(186) Table . . . is the result of a cointegration of a receptor Ti plasmid with an intermediate vector. Each intermediate vector contains a "chimeric" "toxin" gene comprising a plant promotor sequence derived from the indicated expression vector and a Bt gene cassette.

DETD(217) This example describes the construction of pHD205, an intermediate vector containing a "chimeric" Bt2 "toxin" gene comprising: the nopaline synthase promoter, the Bt2 "toxin" gene cassette from pHD160 and a DNA fragment containing the 3' untranslated region of the nopaline synthase gene including the polyadenylation site. In the "chimeric" gene, the Bt2 gene cassette is oriented such that the expression of the Bt2 protein can be obtained from the. . . are fragments of approximately 6200 bp, 3000 bp, 1800 bp and 920 bp. A recombinant plasmid with the alpha-orientation (the "toxin" gene under the control of the nopaline synthase promotor) is used in subsequent experiments and called pHD205.

DETD(219) This example describes the construction of pHD208. The intermediate vector pHD208 contains a "chimeric" Bt2 "toxin" gene comprising: the promotor from a pea gene encoding a small subunit of ribulose

biphosphate carboxylase (Pssu), the Bt2 "toxin" gene cassette from pHD160 and the 3' untranslated region of the octopine synthase gene including the polyadenylation site. The fragments of the "chimeric" gene were assembled in the cloning vector pGV831 as described in this example and as diagrammed in FIG. 29. The. . .

DETD(263) 10. Isolation of plant cells and plants containing the "chimeric" "toxin" gene inserted in their genome

DETD(506) A . . . transformation vectors described herein will contain, stably inserted into their genome, a fragment or newly acquired DNA containing both a "chimeric" Bt "toxin" gene and a marker gene (nos, NPTII). This was confirmed by the results of southern blotting experiments. The new phenotypic traits acquired through this transformation method (expression of Bt "Toxin", antibiotic resistance, nopaline production) will be inherited according to classic Mendelian genetics. To verify stable inheritance of the new traits, F.sub.1 descendants from transformed plants were analysed for the expression of Bt "toxin" and synthesis of nopaline.

DETD(520) TABLE 4

Toxicity of Bt:NPT2 "Fusion" Protein on 3rd Instar P. brassicae (% Mortality After 4 Days)

	"Toxin" dose (ug/ml)				
Bt protein	0.1	0.2	0.3	0.6	1
Bt2	70	NT.sup.(x)		90	NT 100
Bt:NPT2	NT. . .				

DETD(521) TABLE 5

Toxicity of Intact Bt2 Protein, 60 Kd "Processed" Bt2 Protein (Trypsin Digested) and Bt:NPT2 "Fusion" Protein on Larvae of Manduca sexta

% Mortality after 4 days							
"Toxin" dose: (ng/cm.sup.2)	0	0.67	2	6	18	54	162
130 Kd Bt2	0	0	0	0	3	8. . .	0 0 0 60 100
Vt:NPT2	--	0	0	0	0	83	100

Larval Weight after 4 days (mg/larva)					
"Toxin" dose: (ng/cm.sup.2)	0	0.67	2	6	18
130 Kd Bt2	27.4	20.7	9.4	5.4	2.4
60 Kd Bt2 --	16.3	8.3	6.4	3.9	
Bt:NPT2 --	26.5	15.8	7.7	4.5	

"Toxin" dilutions were applied on artificial diet as described in Section 12. Thirty (30) 1st instar larvae were used per. .

US PAT NO: 5,723,756 [IMAGE AVAILABLE] L10: 12 of 31
TITLE: Bacillus "thuringiensis" strains and their genes encoding insecticidal toxins

ABSTRACT: Two new Bacillus "thuringiensis" strains, which are deposited at the DSM under accession numbers 5870 and 5871, produce new crystal proteins during sporulation that. . . from either one of the strains and encodes an insecticidally effective portion of its respective protoxin or encodes its respective "toxin", is resistant to Coleoptera. Each strain, itself, or its crystals, crystal proteins, protoxin, "toxin" and/or insecticidally effective protoxin portion can be used as the active ingredient in an insecticidal composition for combatting Coleoptera.

We claim:
1. A transformed plant cell comprising a "chimeric" gene comprising an isolated DNA sequence encoding a Btl109P protein of SEQ. ID. No. 1; or an insecticidally effective part. . . protein of SEQ. ID. No. 1, or a truncated Btl109P protein of SEQ. ID. No. 1 having at least the "toxin" activity of the Btl109P protein, said DNA being under the control of a plant expressible promoter.

US PAT NO: 5,625,136 [IMAGE AVAILABLE] L10: 13 of 31
ABSTRACT: DNA . . . for expression in plants are disclosed. The DNA sequences preferably encode for an insecticidal polypeptides, particularly insecticidal proteins from Bacillus "thuringiensis". Plant promoters, particular tissue-specific and tissue-preferred promoters are also provided. Additionally disclosed are transformation vectors comprising said DNA sequences. The. . .

BSUM(8) Fischhoff et al., EP 0 385 962 (1990), relates to plant genes encoding the crystal protein "toxin" of Bacillus "thuringiensis". At table V, Fischhoff et al. disclose percent usages for codons for each amino acid. At page 8, Fischoff et. . .

DETD(10) The . . . in maize plants. In a preferred embodiment of the present invention, the DNA sequences encode the production of an insecticidal "toxin", preferably a polypeptide sharing substantially the amino acid sequence of an insecticidal crystal protein "toxin" normally produced by Bacillus "thuringiensis". The synthetic gene may encode a truncated or full-length insecticidal protein. Especially preferred are synthetic DNA sequences which encode a. . . encode a polypeptide having an amino acid sequence essentially the same as one of the crystal protein toxins of Bacillus "thuringiensis" variety kurstaki, HD-1.

DETD(12) The synthetic DNA sequences of the present invention are designed to encode insecticidal proteins from Bacillus "thuringiensis", but are optimized for expression in maize in terms of G+C content and codon usage. For example, the maize codon usage table described in Murray et al., supra, is used to reverse translate the amino acid sequence of the "toxin" produced by the Bacillus "thuringiensis" subsp. kurstaki HD-1 crylA(b) gene, using only the most preferred maize codons. The reverse translated DNA sequence is referred to. . .

DETD(23) In . . . or temperature stable compared to the native crylA(b) protein. It has been shown that the crylA(b) gene found in Bacillus "thuringiensis" kurstaki HD-1 contains a 26 amino acid deletion, when compared with the crylA(a) and crylA(c) proteins, in the --COOH half. . . the protein. This deletion leads to a temperature-sensitive crylA(b) protein. See M. Geiser, EP 0 440 581, entitled "Temperaturstabiles Bacillus "thuringiensis"-"Toxin"". Repair of this deletion with the corresponding region from the crylA(a) or crylA(c) protein improves the temperature stability of the. . .

DETD(39) The . . . Biochem. Biophys. Acta, 939:57-63 (1988); sodium channel proteins and synthetic fragments, Oiki et al. PNAS USA, 85:2395-2397 (1988); the alpha "toxin" of Staphylococcus aureusm Tobkes et al., Biochem., 24:1915-1920 (1985); apolipoproteins and fragments thereof, Knott et al., Science 230:37 (1985); Nakagawa. . . 16:561-581 (1987); lectins, Lis et al., Ann. Rev. Biochem., 55:35-68 (1986); protease and amylase inhibitors; and insecticidal proteins from Bacillus "thuringiensis", particularly the delta-endotoxins from B. "thuringiensis"; and from other bacteria or fungi.

DETD(44) For example, by "fusing" crylA(b) with the pollen and PEP carboxylase promoters, one would obtain expression of this gene in green tissues and pollen. "Fusing" a pith-preferred promoter with the crylB delta "endotoxin" from Bacillus "thuringiensis" would produce expression of this insecticidal protein most abundantly in the pith of a transformed plant, but not in seed. . . burrow into the stalk of the plant after feeding on leaf tissue and/or pollen, it would then encounter the crylB delta-"endotoxin" and be exposed to a second insecticidal component. In this manner, one can differentially express two different insecticdal components in. . .

DETD(280) pCIB932 is a pUC19-based plasmid containing the "chimeric" gene Pep-C:promoter.backslash.Bt.backslash.Pep-C:terminator. It is composed of fragments derived from pPEP-10, a HindIII subclone of a genomic clone, Hi-lambda-14, PNAS USA,. . . photosynthesis, and from pCIB930, which is a BamHI fragment containing the 645 amino acid truncated form of the the crylAb "endotoxin" gene in the BamHI site of pUC18.

DETD(649) pCIB4431 is a vector designed to transform maize. It contains two "chimeric" Bt "endotoxin" genes expressible in maize. These genes are the PEP carboxylase promoter/synthetic-crylA(b) and a pollen promoter/synthetic-crylA(b). The PEP carboxylase/crylA(b) gene in. . .

CLMS(5) 5. The "chimeric" gene of claim 4 wherein said promoter is selected from the group consisting of a CaMV 35S promoter, CaMV 195. . .

CLMS(9) 9. A plant stably transformed with the "chimeric" gene of claim 4.

CLMS(10) 10. A plant stably transformed with the "chimeric" gone of claim 5.

CLMS(14) 14. A maize plant stably transformed with the "chimeric" gene of claim 4.

CLMS(15) 15. A maize plant stably transformed with the "chimeric" gene of claim 5.

US PAT NO: 5,614,395 [IMAGE AVAILABLE] L10: 14 of 31
ABSTRACT: The . . . invention provides chemically regulatable DNA sequences capable of regulating transcription of an associated DNA sequence in plants or plant tissues, "chimeric" constructions containing such sequences, vectors containing such sequences and "chimeric" constructions, and transgenic plants and plant tissues containing these "chimeric" constructions. In one aspect, the chemically regulatable DNA sequences of the invention are derived from the 5' region of genes. . .

BSUM(43) Recent . . . novel approach in the production of crop plants resistant to pests. Most notably, the expression of genes encoding the Bacillus "thuringiensis" .delta.-"endotoxin" has been successful in a wide range of plant species, and the analysis of transgenic lines expressing such genes has. . .

DETD(223) BT Bacillus "thuringiensis" "endotoxin"

DETD(309) A preferred "chimeric" DNA sequence is a two or three component sequence wherein the coding DNA component sequence codes for a phenotypic trait,. . . or female sterility; and production of an enzyme or other reporter compound. Particularly preferred is a two or three component "chimeric" DNA sequence wherein the coding component sequence codes for tolerance or resistance to herbicides, for example codes for wild-type or. . . resistant acetohydroxyacid synthase (AHAS), or wherein the coding component sequence codes for resistance to insects, for example codes for Bacillus "thuringiensis" "endotoxin" (BT).

DETD(310) If the "chimeric" sequence is to be used as an assay for chemical regulators, the phenotypic trait is preferably an assayable marker. Suitable. . . (LUX), chloramphenicol acetyltransferase (CAT), neomycin phosphotransferase (NPT), nopaline synthase (NOS), octopine synthase (OCS), beta-glucuronidase (GUS), acetohydroxyacid synthase (AHAS), and Bacillus "thuringiensis" "endotoxin" (BT). Preferred markers are beta-glucuronidase (GUS), acetohydroxyacid synthase (AHAS), and Bacillus "thuringiensis" "endotoxin" (BT).

DETD(311) A representative example of such a "chimeric" DNA sequence, described in detail in the examples, is a two-part "chimeric" DNA sequence which contains the 5' flanking, non-coding sequence of the PR-1a gene. While one of the exemplified marker is the coding sequence for the GUS gene, any of the above mentioned markers could be used. The analogous three-part "chimeric" sequence contains part of the coding sequence of the PR-1a gene. These constructions are particularly useful because the effect of. . . tobacco .beta.-1,3-glucanase genes and those which comprise the coding sequence for wild-type or herbicide resistant acetohydroxyacid synthase or for Bacillus "thuringiensis" "endotoxin", are described in Part O, Examples.

DETD(342) Further . . . (LUX), chloramphenicol acetyltransferase (CAT), neomycin phosphotransferase (NPT), nopaline synthase (NOS), octopine synthase (OCS), beta-glucuronidase (GUS), acetohydroxyacid synthase (AHAS), and Bacillus "thuringiensis" "endotoxin" (BT).

DETD(345) In . . . (LUX), chloramphenicol acetyltransferase (CAT), neomycin phosphotransferase (NPT), nopaline synthase (NOS), octopine synthase (OCS), beta-glucuronidase (GUS), acetohydroxyacid synthase (AHAS), and Bacillus "thuringiensis" "endotoxin" (BT).

DETD(359) Other . . . (LUX), chloramphenicol acetyltransferase (CAT), neomycin phosphotransferase (NPT), nopaline synthase (NOS), octopine synthase (OCS), beta-glucuronidase (GUS), acetohydroxyacid synthase (AHAS) and Bacillus "thuringiensis" "endotoxin" (Bt) (Williams et al., Bio/Technology, 10: 540-543 (1992). The assay can be performed using whole plants or with plant tissue. . .

DETD(465) Using the vectors of this invention, these clones can then be used for the preparation of "chimeric" genes containing three parts as discussed previously. These "chimeric" DNA sequences contain the chemically regulatable sequence, part of the transcribable DNA and a third DNA sequence from a foreign. . . fragment from the parent gene prior to attachment to a coding sequence from a foreign source to prepare a two-part "chimeric" gene as described above. In a preferred embodiment that part of the "chimeric" gene which is not the chemically regulatable sequence constitutes a reporter gene for an easily observed or detected phenotypic trait. The following examples illustrate the genes for beta-glucuronidase, wild-type and herbicide resistant acetohydroxyacid synthase, and Bacillus "thuringiensis" "endotoxin", but a variety of other-reporter genes can be envisioned as described above. In a further preferred embodiment the coding component DNA sequence of the "chimeric" gene codes for tolerance or resistance to herbicides or for resistance to insects. This embodiment is exemplified by the mentioned genes for acetohydroxyacid synthase and for Bacillus "thuringiensis" "endotoxin".

DETD(664) pCIB10/35Bt(607),. . . sequence coding for approximately 607 amino acids, is prepared from plasmid pCIB10/35Sbt, a plasmid containing the protoxin gene from Bacillus "thuringiensis" "endotoxin". E. coli MC1061 containing pCIB10/355bt was deposited at the American Type Culture Collection, ATCC No. 67329, Feb. 27, 1987. A. . .

DETD(1509) Assay for Chemically Inducible DNA Sequences: Bacillus "thuringiensis" "Endotoxin"

DETD(1513) An . . . plate and this allowed to incubate overnight at 4.degree. C. Antiserum is produced by immunizing rabbits with gradient-purified Bt (Bacillus "thuringiensis" "endotoxin") crystals (Ang, B.J. & Nicke K.W., Appl. Environ. Microbiol. 36: 625-626 (1978)) solubilized with sodium dodecyl sulfate. The plate is. . .

DETD(1697) E. Chemical regulation of a gene encoding the delta-"endotoxin" of Bacillus "thuringiensis" in nahG-expressing plants.

DETD(1698) Plants possessing the PR-1a promoter "fused" to a gene encoding the delta-"endotoxin" of Bacillus "thuringiensis" (Williams et al., Bio/Technology 10: 540-543 (1992) are crossed to nahG-expressing lines nahG-3, -8 and -10. Progeny lines carrying both transgene constructions are found to express the "endotoxin" gene when induced by benzo-1,2,3-thiodiazole-7-carboxylic acid, but not when treated with SA. Further, there is no "endotoxin" gene expression in response to fluctuating endogenous levels of SA as would occur in plants not expressing the nahG gene. . .

CLMS(1) What . . . A method of screening for agrochemicals having the ability to induce SAR in plants, said method comprising: (a) transforming a "chimeric" DNA molecule into a plant or plant part, said "chimeric" DNA molecule comprising: (i) a nucleic acid promoter from the 5' flanking region of a plant pathogenesis-related protein gene inducible. . .

CLMS(11) 11. . . . selected from the group consisting of: luciferase, chloramphenicol acetyltransferase, neomycin phosphotransferase, nopaline synthase, octopine synthase, beta-1,3-glucuronidase, acetohydroxyacid synthase, and Bacillus "thuringiensis" "endotoxin".

US PAT NO: 5,593,881 [IMAGE AVAILABLE] L10: 15 of 31
TITLE: Bacillus "thuringiensis" delta-"endotoxin"

ABSTRACT: An improved Bacillus "thuringiensis" (B.t.) delta-"endotoxin" is created by the modification of the gene encoding the "toxin". The toxicity of a B.t. "toxin" was improved by replacing the native protoxin segment with an alternate protoxin segment by constructing a "chimeric" "toxin" gene.

BSUM(2) The soil microbe Bacillus "thuringiensis" (B.t.) is a Gram-positive, spore-forming bacterium characterized by parasporal crystalline protein inclusions. These inclusions often appear microscopically as distinctively shaped crystals. The proteins can be highly toxic to pests and specific in their toxic activity. Ce B.t. "toxin" genes have been isolated and sequenced, and recombinant DNA-based B.t. products have been produced and approved for use. In addition,. . . approaches for delivering these B.t. endotoxins to agricultural environments are under development, including the use of plants genetically engineered with "endotoxin" genes for insect resistance and the use of stabilized intact microbial cells as B.t. "endotoxin" delivery vehicles (Gaertner, F. H., L. Kim [1988] TIBTECH 6:S4-S7). Thus, isolated B.t. "endotoxin" genes are becoming commercially valuable.

BSUM(3) Until . . . has been largely restricted to a narrow range of lepidopteran (caterpillar) pests. Preparations of the spores and crystals of B. "thuringiensis" subsp. kurstaki have been used for many years as commercial insecticides for lepidopteran pests. For example, B. "thuringiensis" var. kurstaki HD-1 produces a crystalline .delta.-"endotoxin" which is toxic to the larvae of a number of lepidopteran insects.

BSUM(7) A majority of Bacillus "thuringiensis" .delta.-"endotoxin" crystal protein molecules are composed of two functional segments. The protease-resistant core "toxin" is the first segment and corresponds to about the first half of the protein molecule. The three-dimensional structure of a core segment of a crylIIA B.t. .delta.-"endotoxin" is known and it is proposed that all related toxins have that same overall structure (Li, J., J. Carroll, D. . . . this second segment will be referred to herein as the "protoxin segment." The protoxin segment is believed to participate in "toxin" crystal formation (Arvidson, H., P. E. Dunn, S. Strand, A. I. Aronson [1989] Molecular Microbiology 3:1533-1534; Choma, C. T., W. K. Surewicz, P. R. Carey, M. Pozsgay, T. Raynor, H. Kaplan [1990] Eur. J. Biochem. 189:523-527). The full "toxin" molecule is rapidly processed to the resistant core segment by protease in the insect gut. The protoxin segment may thus convey a partial insect specificity for the "toxin" by limiting the accessibility of the core to the insect by reducing the protease processing of the "toxin"

molecule (Haider, M. Z., B. H. Knowles, D. J. Ellar [1986] Eur. J. Biochem. 156:531-540) or by reducing *toxin* solubility (Aronson, A. I., E. S. Han, W. McGaughey, D. Johnson [1991] Appl. Environ. Microbiol. 57:981-986).

BSUM(8) *Chimeric* proteins joined within the *toxin* domains have been reported between CrylC and CrylA(b) (Honee, G., D. Convents, J. Van Rie, S. Jansens, M. Perferoen, B. Visser [1991] Mol. Microbiol. 5:2799-2806); however, the activity of these *chimeric* proteins was either much less, or undetectable, when compared to CrylC on a relevant insect.

BSUM(9) Honee et al. (Honee, G., W. Vriezen, B. Visser [1990] Appl. Environ. Microbiol. 56:823-825) also reported making a *chimeric* *fusion* protein by linking tandem *toxin* domains of CrylC and CrylA(b). The resulting protein had an increased spectrum of activity equivalent to the combined activities of the individual toxins; however, the activity of the *chimeric* was not increased toward any one of the target insects.

BSUM(11) The subject invention concerns the discovery that the activity of a Bacillus *thuringiensis* (B.t.) .delta.-*endotoxin* can be substantially improved by replacing native protoxin amino acids with an alternate protoxin sequence, yielding a *chimeric* *toxin*. In a specific embodiment of the subject invention, a *chimeric* *toxin* is assembled by substituting all or part of the crylA(b) protoxin segment for all or part of the native crylC protoxin segment. The crylC/crylA(b) *chimeric* *toxin* demonstrates an increased toxicity over the crylC/crylC *toxin* produced by the native gene.

BSUM(12) One aspect of the subject invention pertains to genes which encode the advantageous *chimeric* toxins. Specifically exemplified is a gene comprising DNA encoding the crylC core N-terminal *toxin* portion of the *chimeric* *toxin* and the crylA(b) C-terminal protoxin portion of the *toxin*.

BSUM(13) The subject invention further pertains to the use of the *chimeric* *toxin*, or microbes containing the gene encoding the *chimeric* *toxin*, in methods for controlling lepidopteran pests. The subject invention also includes use of the *chimeric* gene encoding the claimed *toxin*. The *chimeric* gene can be introduced into a wide variety of microbial or plant hosts. A transformed host expressing the *chimeric* gene can be used to produce the lepidopteran-active *toxin* of the subject invention. Transformed hosts can be used to produce the insecticidal *toxin* or, in the case of a plant cell transformed to produce the *toxin*, the plant will become resistant to insect attack.

BSUM(14) Still further, the invention includes the treatment of substantially intact recombinant cells producing the *chimeric* *toxin* of the invention. The cells are treated to prolong the lepidopteran activity when the substantially intact cells are applied to. . . nor diminish the cell's capability of protecting the pesticide. The treated cell acts as a protective coating for the pesticidal *toxin*. The *toxin* becomes active upon ingestion by a target insect

DRWDD(5) FIG. 4--The NsiI *toxin*-containing fragment with the new restriction sites is ligated to the vector-containing DNA from pMYC1050.DELTA.BamHI to give pMYC2244. A BamHI-PvuI PCR-derived DNA fragment containing the crylC *toxin* is exchanged for the equivalent fragment in pMYC2244. The resulting *chimera* is called pMYC2238. B=BamHI, C=ClaI, H=HindIII, N=NsiI, P=PvuI

DRWDD(18) SEQ ID NO. 11 shows an amino acid sequence for a *chimeric* *toxin* of the subject invention

DRWDD(19) SEQ ID NO. 12 shows an alternate amino acid sequence for a *chimeric* *toxin* of the subject invention.

DETD(2) The subject invention concerns the discovery of highly active *chimeric* Bacillus *thuringiensis* toxins. These *chimeric* toxins are created by replacing all or part of the native protoxin segment of a full length B.t. *toxin* with an alternate protoxin segment. In a preferred embodiment, the *chimeric* *toxin* comprises a crylA(b) C-terminal protoxin portion and a crylC core N-terminal *toxin* portion. As used herein, reference to a *core* *toxin* portion refers to the portion of the full length B.t. *toxin*, other than the protoxin, which is responsible for the pesticidal activity of the *toxin*.

DETD(6) The . . . that can be carried out according to the subject invention. BamHI and PvuI cloning sites were introduced into a crylA(c)/crylA(b) *chimeric* *toxin* gene by mutagenesis using the PCR technique of Splice Overlap Extension (SOE) (Horton, R. M., H. D. Hunt, S. N. . . pMYC2224. A plasmid created in this manner, pMYC2238, consisted of a short segment of crylA(c) followed by crylC to the *toxin*/protoxin segment junction. The protoxin segment was crylA(b) from pMYC1050. Fragments of plasmid pMYC2238, plasmid pMYCI197, and a crylC portion of plasmid pMYC394 were ligated to construct a *chimeric* gene encoding the *toxin* of the subject invention. The *chimeric* gene encodes the claimed *toxin* comprising a crylC core N-terminal *toxin* portion and a crylA(b) C-terminal protoxin portion which has increased lepidopteran activity compared to a native crylC *toxin*.

DETD(7) The *chimeric* toxins of the subject invention comprise a full core N-terminal *toxin* portion of a B.t. *toxin* and, at some point past the end of the *toxin* portion, the protein has a transition to a heterologous protoxin sequence. The transition to the heterologous protoxin segment can occur at approximately the *toxin*/protoxin junction or, in the alternative, a portion of the native protoxin (extending past the *toxin* portion) can be retained with the transition to the heterologous protoxin occurring downstream. As an example, one *chimeric* *toxin* of the subject invention has the full *toxin* portion of crylC (amino acids 1-616), a portion of the native crylC protoxin (amino acids 617 to 655), and a. . . acids 656 to the C-terminus). In a preferred embodiment, the heterologous portion of the protoxin is derived from a crylA(b) *toxin*.

DETD(8) A. . . certain class such as crylC, will vary to some extent in length and the precise location of the transition from *toxin* portion to protoxin portion. Typically, the crylA(b) and crylC toxins will be about 1150 to about 1200 amino acids in length. The transition from *toxin* portion to protoxin portion will typically occur at between about 50% to about 60% of the full length *toxin*. The *chimeric* *toxin* of the subject invention will include the full expanse of this core N-terminal *toxin* portion. Thus, the *chimeric* *toxin* will comprise at least about 50% of the full length B.t. *toxin*. This will typically be at least about 600 amino acids. With regard to the protoxin portion, the full expanse of the crylA(b) protoxin portion extends from the end of the *toxin* portion to the C-terminus of the molecule. It is the last about 100 to 150 amino acids of this portion which are most critical to include in the *chimeric* *toxin* of the subject invention. In a *chimeric* *toxin* specifically exemplified herein, at least amino acids 1085 to the C-terminus of the crylA(b) molecule are utilized. Thus, it is. . . approximately 5 to 10% of the overall B.t. protein which should comprise heterologous DNA (compared to the cryIF core N-terminal *toxin* portion) included in the *chimeric* *toxin* of the subject invention. Thus, a preferred embodiment of the subject invention is a *chimeric* B.t. *toxin* of about 1150 to about 1200 amino acids in length, wherein the *chimeric* *toxin* comprises a crylC core N-terminal *toxin* portion of at least about 50 to 60% of a full crylC molecule, but no more than about 90 to 95% of the full molecule. The *chimeric* *toxin* further comprises a crylA(b) protoxin C-terminal portion which comprises at least about 5 to 10% of the crylA(b) molecule. The transition from crylC to crylA(b) sequence thus occurs within the protoxin segment (or at the junction of the *toxin* and protoxin segments) between about 50% and about 95% of the way through the molecule. In the specific example provided herein, the transition from the crylC sequence to the crylA(b) sequence occurs prior to amino acid 1085 of the *chimeric* *toxin*.

DETD(9) A specific embodiment of the subject invention is the *chimeric* *toxin* of SEQ ID NO. 11. Other constructs may be made and used by those skilled in this art having the benefit of the teachings provided herein. The core *toxin* segment of cryI proteins characteristically ends with the sequence: Val/Leu Tyr/Ile Ile Asp Arg/Lys Ile/Phe Glu Ile/Phe Ile/Leu/Val Pro/Leu Ala/Val. . . protoxin segments of the cryI toxins (following residue 616 of SEQ ID NO. 11) bear more sequence similarity than the *toxin* segments. Because of this sequence similarity, the transition point in the protoxin segment for making a *chimeric* protein between the crylC sequence and the crylA(b) sequence can be readily determined by one skilled in the art. From. . .

DETD(10) Therefore a *chimeric* *toxin* of the subject invention can comprise the full crylC *toxin* and a portion of the crylC protoxin, transitioning to the corresponding crylA(b) sequence at any position between the end of the *toxin* segment (as defined above) and about position 1084. Preferably, the amino acids which correspond to positions 1085 through 1190 (SEQ. . .

DETD(14) The subject invention not only includes the novel *chimeric* toxins and the genes encoding these toxins but also includes uses of these novel toxins and genes. For example, the. . . of the subject invention may be used to transform host cells. These host cells expressing the gene and producing the *chimeric* *toxin* may be used in insecticidal compositions or, in the case of a transformed plant cell, in conferring insect resistance to. . .

DETD(18) A. . . for determining in a known manner whether hybridization has occurred. Such a probe analysis provides a rapid method for identifying *toxin*-encoding genes useful according to the subject invention. Preferably, such genes would be crylC genes whose core *toxin*-encoding N-terminal portions can be used with a crylA(b) protoxin-encoding C-terminal portion to create a *chimeric* gene according to the subject invention. The nucleotide segments which are used as probes according to the invention can be. . .

DETD(19) Certain *chimeric* toxins of the subject invention have been specifically exemplified herein. It should be readily apparent that the subject invention comprises. . . variant or equivalent toxins (and nucleotide sequences encoding equivalent toxins) having the same or similar pesticidal activity of the exemplified *toxin*. Equivalent toxins will have amino acid homology with the exemplified *toxin*. This amino acid homology will typically be greater than 75%, preferably be greater than 90%, and most preferably be greater than 95%. The amino acid homology will be highest in critical regions of the *toxin* which account for biological activity or are involved in the determination of three-dimensional configuration which ultimately is responsible for the. . .

DETD(22) Recombinant Hosts. A gene encoding the *chimeric* toxins of the subject invention can be introduced into a wide variety of microbial or plant hosts. Expression of the *toxin* gene results, directly or indirectly, in the intracellular production and maintenance of the pesticidal *chimeric* *toxin*. With suitable microbial hosts, e.g., Pseudomonas, the microbes can be applied to the situs of the pest, where they will proliferate and be ingested. The result is control of the pest. Alternatively, the microbe hosting the *toxin* gene can be treated under conditions that prolong the activity of the *toxin* and stabilize the cell. The treated cell, which retains the toxic activity, then can be applied to the environment of. . .

DETD(23) Where the gene encoding the *chimeric* *toxin* is introduced via a suitable vector into a microbial host, and said host is applied to the environment in a. . .

DETD(25) A wide variety of ways are available for introducing a gene encoding a *chimeric* *toxin* into a microorganism host under conditions which allow for the stable maintenance and expression of the gene. These methods are. . .

DETD(26) Treatment of cells. As mentioned above, recombinant cells producing the *chimeric* *toxin* of the subject invention can be treated to prolong the toxic activity and stabilize the cell. The pesticide microcapsule that is formed comprises the B.t. *toxin* within a cellular structure that has been stabilized and will protect the *toxin* when the microcapsule is applied to the environment of the target pest. Suitable host cells may include either prokaryotes or. . .

DETD(28) Treatment of the microbial cell, e.g., a microbe containing the gene encoding a *chimeric* *toxin* of the subject invention, can be by chemical or physical means, or by a combination of chemical and/or physical means, so long as the technique does not deleteriously affect the properties of the *toxin*, nor diminish the cellular capability of protecting the *toxin*. Examples of chemical reagents are halogenating agents, particularly halogens of atomic no. 17-80. More particularly, iodine can be used under. . . and Company, 1967); or a combination of physical (heat) and chemical agents that preserve and prolong the activity of the *toxin* produced in the cell when the cell is administered to the host environment. Examples of physical means are short wavelength. . .

DETD(31) Growth of cells. The cellular host containing the gene encoding a *chimeric* *toxin* of the subject invention may be grown in any convenient nutrient medium, where the DNA construct provides a selective advantage,. . .

DETD(32) Formulations. Recombinant microbes comprising the gene encoding the *chimeric* *toxin* disclosed herein, can be formulated into bait granules and applied to the soil. Formulated product can also be applied as. . .

DETD(43) A. . . the vector construction may be found in EPO patent application 0 471 564. Plasmid DNA of pMYC1050 initially contained the *chimeric* *toxin* gene crylA(c)/crylA(b). The *toxin* encoded by this gene is described in U.S. Pat. No. 5,055,294. pMYC1050 was constructed by re-cloning the *toxin* gene and promoter of pM3,130-7 (disclosed in U.S. Pat. No. 5,055,294) into a pTJS260-based-vector such as pMYC467 (disclosed in U.S. Pat. No. 5,169,760) by methods well known in the art. In particular, the pM3,130-7 promoter and *toxin* gene can be obtained as a BamHI to NdeI fragment and placed into the pMYC467 plasmid, replacing a fragment bounded. . .

DETD(56) Example 4--Activity of the *Chimeric* *Toxin* Against Spodoptera exigua

DETD(57) Serial . . . 3-ml wells (Nutrend Container Corporation, Jacksonville, Fla.). Water served as a control as well as the vehicle to introduce the *toxin* protein into the diet. Second-instar Spodoptera exigua larvae were placed singly onto the diet mixture. Wells were then sealed with. . . or four days, respectively. LC.sub.50 s were determined by standard log-probit analysis (POLO-PC, LeOra Software, 1987). CrylC and the crylC/crylA(b) *chimeric* were tested simultaneously and representative results are as follows:

DETD(59) Example 5--Insertion of the Gene Encoding the *Chimeric* *Toxin* Into Plants

DETD(61) The gene encoding the *chimeric* *toxin*, as disclosed herein, can be inserted into plant cells using a variety of techniques which are well known in the. . . higher plants. The vectors comprise, for example, pBR322, pUC series, M13mp series, pACYC184, etc. Accordingly, the sequence encoding the B.t. *toxin* can be inserted into the vector at a suitable restriction site. The resulting plasmid is used for transformation into E.. . .

DETD(67) Example 6--Cloning of the Gene Encoding the *Chimeric* *Toxin* Into Insect Viruses

DETD(68) A. . . genome of the insect virus, thus enhancing the pathogenicity of the virus. Methods for constructing insect viruses which comprise the *chimeric* *toxin* gene are well known and readily practiced by those skilled in the art. These procedures are described, for example, in. . .

CLMS(1) We claim: 1. An isolated DNA molecule comprising a nucleotide sequence encoding a *chimeric* Bacillus *thuringiensis* *toxin* of approximately 1150 to 1200 amino acids, wherein said *toxin* comprises a core N-terminal *toxin* portion having a sequence of at least about 600 amino acids and no more than about 1100 amino acids, wherein the amino acid sequence from the end of said core N-terminal sequence to the C-terminus of the *chimeric* *toxin* is a crylA(b) C-terminal protoxin portion having a crylA(b) sequence.

CLMS(7) 7. A recombinant host transformed to express a *chimeric* Bacillus *thuringiensis* *toxin* comprising a crylC core N-terminal *toxin* portion and a crylA(b) C-terminal protoxin portion.

US PAT NO: 5,545,565 [IMAGE AVAILABLE] L10: 16 of 31

TITLE: Transformation vectors allowing expression of foreign polypeptide endoxins from Bacillus *thuringiensis* in plants

ABSTRACT: Novel transformation vectors containing novel *chimeric* genes allow the introduction of exogenous DNA fragments coding for polypeptide toxins produced by Bacillus *thuringiensis* or having substantial sequence homology to a gene coding for a polypeptide *toxin* as described herein and expression of the *chimeric* gene in plant cells and their progeny after integration into the plant cell genome. Transformed plant cells and their progeny exhibit stably inherited polypeptide *toxin* expression useful for protecting said plant cells and their progeny against certain insect pests and in controlling said insect pests.

BSUM(2) This . . . the use of genetic engineering techniques in the modification of plants. More particularly, it concerns introduction and integration of a *chimeric* gene coding for a polypeptide *toxin* produced by Bacillus *thuringiensis* or having substantial sequence homology to a *toxin* gene described below in plant cells and obtaining an insect controlling level of expression of said polypeptide *toxin* intracellularly by transformed plant cells and their progeny.

BSUM(7) Bacillus *thuringiensis* (referred to at times herein as B.t.) bacteria includes approximately 19 known varieties that produce polypeptide toxins which form parasporal. . . by insect larvae, the crystals are solubilized and processed in the insect midgut to yield at least one active polypeptide *toxin* which is believed to act on the midgut cell membrane. Studies have revealed that individual crystal polypeptides exhibit insecticidal activity.

BSUM(12) It is one object of this invention to provide novel *chimeric* genes coding for the polypeptide produced by Bacillus *thuringiensis*, or coding for a polypeptide *toxin* having substantial sequence homo a *toxin* gene described herein. The *chimeric* genes' plant regulatory sequences direct expression in transformed plant cells.

BSUM(19) (b) at least one DNA fragment coding for a polypeptide *toxin* produced by Bacillus *thuringiensis* or having substantial sequence homology thereto.

BSUM(26) (ii) at least one DNA fragment coding for a polypeptide *toxin* produced by Bacillus *thuringiensis* or at least one DNA fragment having substantial sequence homology thereto.

BSUM(30) (b) at least one DNA fragment coding for a polypeptide *toxin* produced by Bacillus *thuringiensis*, or at least one DNA fragment having substantial sequence homology thereto.

BSUM(34) Transformed plant cells and their progeny intracellularly express a polypeptide *toxin* substantially similar to the polypeptide toxins produced by Bacillus *thuringiensis* and are substantially toxic to certain insects. Transformed plant cells and their progeny may be used in controlling said insects.

DETD(7) (1) Isolation of at least one DNA fragment from Bacillus *thuringiensis* coding for a polypeptide *toxin* by digestion of bacterial DNA and inserting the mixture of DNA fragments obtained into a cloning vehicle harbored in a. . .

DETD(24) Transformed plant cells and their progeny should express a polypeptide "toxin" substantially similar to polypeptide toxins being produced by Bacillus "thuringiensis" or a DNA fragment having substantial sequence homology to Bt2.

DETD(67) Straight promotor-gene "fusions" in which only part of the Bt2 coding sequence is used ("truncated Bt2"). Fragments of the Bt2 sequence still encoding an active "toxin" are inserted behind the plant specific promotors: The toxic polypeptides produced in the plant cells using these constructs should have. . .

DETD(70) Straight promotor-gene "fusions" in which a Bt:NPTII "fusion" gene (also referred to at times at Bt2:NPTII) is inserted behind the promotor. "Fusion" genes were constructed, consisting of a fragment of the Bt2 coding sequence (still encoding an active "toxin") "fused" to the coding sequence of the NPTII enzyme. The Bt:NPTII "fusion" genes used here, specify stable "fusion" proteins comprising amino terminal parts of the Bt2 protein "fused" to an intact Neomycin phosphotransferase (NTPII) enzyme. These "fusion" proteins have a specific toxicity comparable to the intact Bt2 protein and retain neomycin phosphotransferase enzyme activity. Thus, expression of the Bt:NPTII "fusion" proteins in plant cells allows direct selection for the production of this protein by isolating Kanamycin resistant (Km.sup.R) transformed cells.. . . to a high level of Kanamycin should identify, among all possible transformations, those which produce high levels of the toxic "fusion" protein. Further, expression of the "fusion" protein by a Bt:NPTII "fusion" gene might have other desirable properties such as stability in plant cells; for example, mRNA may be more stable. Differences in results obtained with these Type IV "fusion" genes might be due to intrinsic differences in the properties of the "fusion" protein expressed as compared to the intact Bt2 protein.

DETD(87) Kronstad et al., J. Bacteriol., 54, p. 419-428 (1983) reported that B.t. berliner 1715 contains two related "toxin" genes which are both located on plasmids. Intact "endotoxin" genes were isolated from a gene bank from total B.t. berliner 1715 plasmid DNA using partial Sau3A digests of plasmid. . . DNA. The pEcoR251 plasmid is a derivative of plasmid pBR322 in which the EcoRI-PuvII fragment has been replaced by a "chimeric" EcoRI endonuclease gene which is "fused" to a P.sub.R promotor fragment derived from plasmid pLK5 (Zabeau and Stanley, EMBO Journal, 1, 1217-1224 (1982)) as depicted in. . .

DETD(134) The previous data suggests that the smallest gene fragment of Bt2, encoding an active "toxin" is contained within the KpnI deletion fragment but extends further than the HindIII site. To map the exact endpoint of the minimal fragment coding for the active "toxin", deletion mutants were constructed which contained N-terminal fragments of decreasing size. To achieve this, we used a strategy which allowed us to construct simultaneously deletion-mutants and translational "fusions" to the NPTII-gene (see Section 7.2.2). The construction of the intermediate plasmid pLBKm25 is outlined in FIG. 18. As shown. . .

DETD(135) As . . . Bal31, cut with SalI, treated with Klenow polymerase and religated (FIG. 19). In this way, the deleted coding region is "fused" to a stopcodon with a minimum of nonsense coding sequence. An overview of the deletion clones is given in FIG.. . . blotting and ELISA for the quantitative detection of Bt2-like polypeptides and in an insect toxicity assay to screen for active "toxin". The results are presented in FIG. 21 and indicate that detection of a stable polypeptide decreases gradually when the endpoint. . .

DETD(140) Since NPTII is a most suitable selection marker in plant engineering, such gene "fusions" could have very promising applications. Indeed when using such NPTII "fusion" proteins to transform plants, a selection for high kanamycin resistance would allow direct selection for a high expression of the "fusion" product. Therefore, "toxin" gene "fusions" with NPTII might be used to transform plants and select for transformed plants expressing high levels of "toxin", by selection for kanamycin resistance.

DETD(169) Previous . . . on the identification of minimal active toxic fragments have shown that this Kpn fragment comprises a (approximately 60 Kd) active "toxin" which exhibits the complete toxic activity of the Bt2 molecule. In the following, we wanted to determine whether the Bt:NPT2 "fusion" protein had still the same degree of toxicity.

DETD(175) 145 . . . concentrations. 8 transformants proved more resistant and were able to grow on concentrations higher than 200 ug/ml of kanamycin. The "fusion" point in all 8 clones was determined by restriction enzyme mapping with an accuracy of 20 bp. Surprisingly 7 out of 8 clones had their "fusion" point around the HindIII site at position 1680 of the Bt gene. One clone (pLBKm860) mapped at position approximately 2050. Although the majority of the deletions were "fused" around position 1800, none of these conferred a higher kanamycin resistant phenotype. The 7 clones which have their "fusion" point positioned around the HindIII site are too short to encode an active "toxin". However, one of the clones (pLBKm860) was:

DETD(185) Table . . . is the result of a cointegration of a receptor Ti plasmid with an intermediate vector. Each intermediate vector contains a "chimeric" "toxin" gene comprising a plant promotor sequence derived from the indicated expression vector and a Bt gene cassette.

DETD(218) This example describes the construction of pHD205, an intermediate vector containing a "chimeric" Bt2 "toxin" gene comprising: the nopaline synthase promotor, the Bt2 "toxin" gene cassette from pHD160 and a DNA fragment containing the 3' untranslated region of the nopaline synthase gene including the polyadenylation site. In the "chimeric" gene the Bt2 gene cassette is oriented such that the expression of the Bt2 protein can be obtained from the. . . are fragments of approximately 6200 bp, 3000 bp, 1800 bp and 920 bp. A recombinant plasmid with the alpha-orientation (the "toxin" gene under the control of the nopaline synthase promotor) is used in subsequent experiments and called pHD205.

DETD(220) This example describes the construction of pHD208. The intermediate vector pHD208 contains a "chimeric" Bt2 "toxin" gene comprisimg the promotor from a pea gene encoding a small subunit of ribulose biphosphate carboxylase (Pssu), the Bt2 "toxin" gene cassette from pHD160 and the 3' untranslated region of the octopine synthase gene including the polyadenylation site. The fragments of the "chimeric" gene were assembled in the cloning vector pGV831 as described in this example and as diagrammed in FIG. 29. The. . .

DETD(264) 10. Isolation of plant cells and plants containing the "chimeric" "toxin" gene inserted in their genome

DETD(496) A . . . transformation vectors described herein will contain, stably inserted into their genome, a fragment of newly acquired DNA containing both a "chimeric" Bt "toxin" gene and a marker gene (nos, NPTII). This was confirmed by the results of southern blotting experiments. The new phenotypic traits acquired through this transformation method (expression of Bt "Toxin", antibiotic resistance, nopaline production) will be inherited according to classic Mendelian genetics. To verify stable inheritance of the new traits, F.sub.1 descendants from transformed plants were analysed for the expression of Bt "toxin" and synthesis of nopaline.

DETD(510) TABLE 4

Toxicity of Bt:NPT2 "Fusion" Protein on 3rd Instar P. brassicae (% Mortality After 4 Days) "Toxin" dose (ug/ml)

Bt protein	0.1	0.2	0.3	0.6	1
Bt2 70 NT.sup.(x)				90	NT 100
Bt:NPT2 NT. . .					

DETD(511) TABLE 5

Toxicity of Intact Bt2 Protein, 60 Kd "Processed" Bt2 Protein (Trypsin Digested) and Bt:NPT2 "Fusion" Protein on Larvae of Manduca sexta

% Mortality after 4 days "Toxin" dose: (ng/cm.sup.2)	0	0.67	2	6	18	54	162
130 Kd Bt2	0	0	0	0	3	8	100. . .
60 Kd Bt2	--	16.3	8.3	6.4	3.9		
Bt:NPT2	--	26.5	15.8	7.7	4.5		

"Toxin" dilutions were applied on artificial diet as described in Section 12. Thirty (30) 1st instar larvae were used per. . .

We claim:

1. A "chimeric" gene comprising: (1) a DNA fragment encoding an insecticidal Bacillus "thuringiensis" Bt2 "toxin" of about 60 to about 80 kD, wherein said Bt2 "toxin" comprises the amino acid sequence of SEQ ID No. 1 from amino acid position 29 to amino acid position 607;. . .

CLMS(2) 2. The "chimeric" gene as defined in claim 1, wherein said Bt2 "toxin" comprises the amino acid sequence of SEQ ID No. 1 from amino acid position 1 to an amino acid position. . .

CLMS(3) 3. The "chimeric" gene as defined in claim 1, wherein said Bt2 "toxin" comprises the amino acid sequence of SEQ ID No. 1 from amino acid position 29 to an amino acid position. . .

CLMS(4) 4. The "chimeric" gene as defined in claim 1, wherein said Bt2 "toxin" comprises the amino acid sequence of SEQ ID No. 1 from amino acid position 1 to amino acid position 607.

CLMS(5) 5. The "chimeric" gene as defined in claim 1, wherein said Bt2 "toxin" comprises the amino acid sequence of SEQ ID No. 1 from amino acid position 1 to amino acid position 725.

CLMS(6) 6. The "chimeric" gene as defined in claim 1, wherein said Bt2 "toxin" comprises the amino acid sequence of SEQ ID No. 1 from amino acid position 29 to amino acid position 725.

CLMS(7) 7. The "chimeric" gene as defined in claim 1, wherein said DNA fragment is artificially made.

CLMS(8) 8. The "chimeric" gene as defined in any of claims 2 to 6, wherein said DNA fragment is artificially made.

CLMS(9) 9. A "chimeric" gene comprising: (1) a DNA fragment encoding an insecticidal Bacillus "thuringiensis" Bt2 "toxin" of about 60 to about 80 kD, wherein said DNA fragment comprises the DNA sequence of SEQ ID No. 1. . .

CLMS(10) 10. The "chimeric" gene as defined in claim 9, wherein said DNA fragment encoding an insecticidal Bacillus "thuringiensis" Bt2 "toxin" of about 60 to about 80 kD comprises the sequence of SEQ ID No. 1 from nucleotide position 141 to. . .

CLMS(11) 11. The "chimeric" gene as defined in claim 9, wherein said DNA fragment encoding an insecticidal Bacillus "thuringiensis" Bt2 "toxin" of about 60 to about 80 kD comprises the sequence of SEQ ID No. 1 from nucleotide position 225 to. . .

CLMS(12) 12. The "chimeric" gene as defined in claims 1 or 9, wherein said promoter region is from a ribulose bisphosphate carboxylase small subunit. . .

CLMS(13) 13. The "chimeric" gene as defined in claims 1 or 9, wherein said promoter region regulates tissue-specific or inducible expression in a plant.

CLMS(14) 14. The "chimeric" gene as defined in claim 1 or 9, which further comprises a 3' untranslated region, including a polyadenylation site, of. . .

CLMS(15) 15. The "chimeric" gene as defined in claim 14, wherein said 3' untranslated end, including a polyadenylation site, is from an octopine synthase.

. . US PAT NO: 5,527,883 [IMAGE AVAILABLE] L10: 17 of 31

ABSTRACT: Bacillus "thuringiensis" "endotoxin" expression in Pseudomonads can be improved by modifying the gene encoding the Bacillus "thuringiensis" "endotoxin". "Chimeric" genes are created by replacing the segment of the Bacillus "thuringiensis" gene encoding a native protoxin with a segment encoding a different protoxin. Exemplified herein is the cryIF/cryI(b) "chimera" wherein the native cryIF protoxin segment has been substituted by the cryIA(b) protoxin segment, to yield improved expression of the cryIF "toxin" in Pseudomonads. The invention also concerns novel genes and plasmids.

BSUM(2) The soil microbe Bacillus "thuringiensis" (B.t.) is a Gram-positive, spore-forming bacterium characterized by parasporal crystalline protein inclusions. These inclusions often appear microscopically as distinctively shaped crystals. The proteins can be highly toxic to pests and specific in their toxic activity. Certain B.t. "toxin" genes have been isolated and sequenced, and recombinant DNA-based B.t. products have been produced and approved for use. In addition,. . . approaches for delivering these B.t. endotoxins to agricultural environments are under development, including the use of plants genetically engineered with "endotoxin" genes for insect resistance and the use of stabilized intact microbial cells as B.t. "endotoxin" delivery vehicles (Gaertner, F. H., L. Kim [1988] TIBTECH 6:S4-S7). Thus, isolated B.t. "endotoxin" genes are becoming commercially valuable.

BSUM(3) Until . . . has been largely restricted to a narrow range of lepidopteran (caterpillar) pests. Preparations of the spores and crystals of B. "thuringiensis" subsp. kurstaki have been used for many years as commercial insecticides for lepidopteran pests. For example, B. "thuringiensis" var. kurstaki HD-1 produces a crystalline .delta.-"endotoxin" which is toxic to the larvae of a number of lepidopteran insects.

BSUM(7) A majority of Bacillus "thuringiensis" .delta.-"endotoxin" crystal protein molecules are composed of two functional segments. The protease-resistant core "toxin" is the first segment and corresponds to about the first half of the protein molecule. The three-dimensional structure of a core segment of a cryIIA B.t. .delta.-"endotoxin" is known and it is proposed that all related toxins have that same overall structure (Li, J., J. Carroll, D.. . . this second segment will be referred to herein as the "protoxin segment." The protoxin segment is believed to participate in "toxin" crystal formation (Arvidson, H., P. E. Dunn, S. Strand, A. I. Aronson [1989] Molecular Microbiology 3:1533-1534; Choma, C. T., W.. . . K. Surewicz, P. R. Carey., M. Pozsgay, T. Raynor, H. Kaplan [1990] Eur. J. Biochem. 189:523-527). The full 130 kDa "toxin" molecule is rapidly processed to the resistant core segment by protease in the insect gut. The protoxin segment may thus convey a partial insect specificity for the "toxin" by limiting the accessibility of the core to the insect by reducing the protease processing of the "toxin" molecule (Haider, M. Z., B. H. Knowles, D. J. Ellar [1986] Eur. J. Biochem. 156:531-540) or by reducing "toxin" solubility (Aronson, A. I., E. S. Han, W. McGaughey, D. Johnson [1991] Appl. Environ. Microbiol. 57:981-986).

BSUM(8) "Chimeric" proteins joined within the "toxin" domains have been reported between CryIC and CryIA(b) (Honee, G., D. Convents, J. Van Rie, S. Jansens, M. Perferoen, B. Visser [1991] Mol. Microbiol. 5:2799-2806); however, the activity of these "chimeric" proteins was either much less, or undetectable, when compared to CryIC on a relevant insect.

BSUM(9) Honee et al. (Honee, G., W. Vriezen, B. Visser [1990] Appl. Environ. Microbiol. 56:823-825) also reported making a "chimeric" "fusion" protein by linking tandem "toxin" domains of CryIC and CryIA(b). The resulting protein had an increased spectrum of activity equivalent to the combined activities of the individual toxins; however, the activity of the "chimeric" was not increased toward any one of the target insects.

BSUM(11) The subject invention concerns the discovery that expression of Bacillus "thuringiensis" (B.t.) .delta.-"endotoxin" in Pseudomonas can be substantially improved by modifying the gene which encodes the B.t. "toxin". Specifically, B.t. "endotoxin" expression in P. fluorescens can be improved by reconstructing the gene so as to replace the native protoxin-encoding segment with an alternate protoxin segment, yielding a "chimeric" gene.

BSUM(12) In specific embodiments of the subject invention, "chimeric" genes can be assembled that substitute a heterologous protoxin segment for a native cryIF protoxin segment. In particular, all or. . . can be used in place of all or part of the region which encodes the protoxin for a native cryIF "toxin". Similarly, a "chimeric" gene can be constructed wherein the region encoding all or part of the protoxin of a cryIF "toxin" is replaced by DNA encoding all or part of the protoxin of a cryIA(c)/cryIA(b) "chimeric" gene. In a specific embodiment, the cryIA(c)/cryIA(b) "chimeric" gene is that which has been denoted 436 and which is described in U.S. Pat. No. 5,128,130. This gene can. . .

BSUM(13) The subject invention also includes use of the "chimeric" gene encoding the claimed "toxin". The "chimeric" gene can be introduced into a wide variety of microbial or plant hosts. A transformed host expressing the "chimeric" gene can be used to produce the lepidopteran-active "toxin" of the subject invention. Transformed hosts can be used to produce the insecticidal "toxin" or, in the case of a plant cell transformed to produce the "toxin", the plant will become resistant to insect attack. The subject invention further pertains to the use of the "chimeric" "toxin", or hosts containing the gene encoding the "chimeric" "toxin", in methods for controlling lepidopteran pests.

BSUM(14) Still further, the invention includes the treatment of substantially intact recombinant cells producing the "chimeric" "toxin" of the invention. The cells are treated to prolong the lepidopteran activity when the substantially intact cells are applied to. . . nor diminish the cell's capability of protecting the pesticide. The treated cell acts as a protective coating for the pesticidal "toxin". The "toxin" becomes active upon ingestion by a target insect

DRWDD(5) FIG. 4--The NsiI "toxin"-containing fragment with the new restriction sites is ligated to the vector-containing DNA from pMYC1050.DELTA.BamHI to give pMYC2224. A BamHI-PvuI PCR-derived DNA fragment containing the cryIF "toxin" is exchanged for the equivalent fragment in pMYC2224. The resulting "chimera" is called pMYC2239. B=BamHI, C=ClaI, H=HindIII, N=NsiI, P=PvuI

DRWDD(6) FIG. 5--The small ApaI DNA fragment of pMYC2047 is substituted for the homologous region of pMYC2239 to give plasmid pMYC2244. This "chimera" consists of cryIF in the "toxin" region and crylA(b) in the protoxin. C=ClaI, H=HindIII, N=NsiI, P=PvuI

DRWDD(9) FIG. 8--A "chimeric" "toxin" containing the 436 protoxin is constructed by substituting a PCR-generated PvuI-BstEII protoxin DNA for the homologous fragment in pMYC2523. The. . .

DETD(23) SEQ ID NO. 22 shows the "toxin"-encoding DNA sequence of pMYC2244, which encodes a cryIF/cryIA(b) "chimeric" "toxin".

DETD(24) SEQ ID NO. 23 shows the predicted amino acid sequence of the cryIF/cryIA(b) "chimeric" "toxin" encoded by pMYC2244.

DETD(27) SEQ ID NO. 26 shows the "toxin"-encoding DNA sequence of pMYC2523, which encodes a cryIF/cryIA(b) "chimeric" "toxin" with codon rework.

DETD(29) SEQ ID NO. 28 shows the "toxin"-encoding DNA sequence of pMYC2254, which encodes a cryIF/436 "chimeric" "toxin".

DETD(37) The subject invention concerns the discovery that certain "chimeric" genes encoding B.t. toxins have improved expression in recombinant Pseudomonas fluorescens. The "chimeric" genes encode toxins wherein all or part of the native protoxin portion has been replaced with all or part of the protoxin from another B.t. "toxin". Specifically exemplified herein are genes which encode a B.t. "toxin" which consists essentially of a cryIF core N-terminal "toxin" portion attached to a protoxin segment which is derived from either a cryIA(b) "toxin" or a cryIA(c)/cryIA(b) "toxin" as described herein. As used herein, reference to a "core" "toxin" portion refers to the portion of the full length B.t. "toxin", other than the protoxin, which is responsible for the pesticidal activity of the "toxin".

DETD(41) The . . . that can be carried out according to the subject invention. BamHI and PvuI cloning sites can be introduced into acrylA(c)/cryIA(b) "chimeric" "toxin" gene by mutagenesis using the PCR technique of Splice Overlap Extension (SOE) (Horton, R. M., H. D. Hunt, S. N.. . . pMYC2224. The new plasmid, which we designated pMYC2239, consisted of a short segment of crylA(c) followed by cryIF to the "toxin"/protoxin segment junction. Thus, the protoxin segment was now derived from crylA(b) (pMYC1050). An ApaI fragment derived from the cryIF clone. . . substituted for the ApaI fragment in pMYC2239. The resulting clone (pMYC2244) consisted of cryIF from the initiator methionine to the "toxin"/protoxin segment junction and crylA(b) to the end of the coding region. Clone pMYC2243 was constructed by SOE to introduce silent. . . from pMYC2243 that contained the silent changes was substituted for the ApaI fragment in pMYC2244 to give clone pMYC2523. The "chimeric" pMYC2523 showed an expression improvement over pMYC2243, which contains unchanged cryIF protein sequence.

DETD(43) The "chimeric" toxins of the subject invention comprise a full core N-terminal "toxin" portion of a B.t. "toxin" and, at some point past the end of the "toxin" portion, the protein has a transition to a heterologous protoxin sequence. The transition to the heterologous protoxin segment can occur at approximately the "toxin"/protoxin junction or, in the alternative, a portion of the native protoxin (extending past the "toxin" portion) can be retained with the transition to the heterologous protoxin occurring downstream. As an example, one "chimeric" "toxin" of the subject invention has the full "toxin" portion of cryIF (amino acids 1-601) and a heterologous protoxin (amino acids 602 to the C-terminus). In a preferred embodiment, the heterologous portion of the protoxin is derived from a cryIA(b) or 436 "toxin".

DETD(44) A . . . certain class such as cryIF, will vary to some extent in length and the precise location of the transition from "toxin" portion to protoxin portion. Typically, the cryIA(b) and cryIF toxins are about 1150 to about 1200 amino acids in length. The transition from "toxin" portion to protoxin portion will typically occur at between about 50% to about 60% of the full length "toxin". The "chimeric" "toxin" of the subject invention will include the full expanse of this core N-terminal "toxin" portion. Thus, the "chimeric" "toxin" will comprise at least about 50% of the full length cryIF B.t. "toxin". This will typically be at least about 590 amino acids. With regard to the protoxin portion, the full expanse of the cryIA(b) protoxin portion extends from the end of the "toxin" portion to the C-terminus of the molecule. It is the last about 100 to 150 amino acids of this portion which are most critical to include in the "chimeric" "toxin" of the subject invention. In a "chimeric" "toxin" specifically exemplified herein, at least amino acids 1043 (of SEQ ID NO. 23) to the C-terminus of the crylA(b) molecule. . . marks the location in the protoxin segment of the molecule beyond which heterologous amino acids will always occur in the "chimeric" "toxin". In another example, the peptide shown as SEQ ID NO. 31 occurs at amino acids 1061 to 1068. In this. . . approximately 5 to 10% of the overall B.t. protein which should comprise heterologous DNA (compared to the cryIF core N-terminal "toxin" portion) in the "chimeric" "toxin" of the subject invention. In the specific examples contained herein, heterologous protoxin sequences occur from amino acid 640 to the. . .

DETD(45) Thus, a preferred embodiment of the subject invention is a "chimeric" B.t. "toxin" of about 1150 to about 1200 amino acids in length, wherein the "chimeric" "toxin" comprises a cryIF core N-terminal "toxin" portion of at least about 50 to 60% of a full cryIF molecule, but no more than about 90 to 95% of the full molecule. The "chimeric" "toxin" further comprises a crylA(b) or a 436 protoxin C-terminal portion which comprises at least about 5 to 10% of the. . . transition from cryIF to crylA(b) or 436 sequence thus occurs within the protoxin segment (or at the junction of the "toxin" and protoxin segments) between about 50% and about 95% of the way through the molecule. In the specific examples provided. . .

(46) A specific embodiment of the subject invention is the "chimeric" "toxin" shown in FIG. 9. Other constructs may be made and used by those skilled in this art having the benefit of the teachings provided herein. The core "toxin" segment of cryI proteins characteristically ends with the sequence: Val/Leu Tyr/Ile Ile Asp Arg/Lys Ile/Phe Glu Ile/Phe/Leu Ile/Leu/Val Pro/Leu Ala/Val. . . NO. 23. Additionally, the protoxin segments of the cryI toxins (which follow residue 601) bear more sequence similarity than the "toxin" segments. Because of this sequence similarity, the transition point in the protoxin segment for making a "chimeric" protein between the cryIF sequence and the crylA(b) or 436 sequence can be readily determined by one skilled in the. . .

(47) Therefore a "chimeric" "toxin" of the subject invention can comprise the full cryIF "toxin" and a portion of the cryIF protoxin, transitioning to the corresponding crylA(b) or 436 sequence at any position between the end of the "toxin" segment (as defined above) and the end of the peptide sequence shown in SEQ ID NO. 31. Preferably, the amino acid sequence of the C-terminus of the "chimeric" "toxin" comprises a crylA(b) sequence or a sequence from the 436 gene or an equivalent of one of these sequences. DETD(51) The subject invention not only includes the novel "chimeric" toxins and the genes encoding these toxins but also includes uses of these novel toxins and genes. For example, a. . . of the subject invention may be used to transform host cells. These host cells expressing the gene and producing the "chimeric" "toxin" may be used in insecticidal compositions or, in the case of a transformed plant cell, in conferring insect resistance to. . .

(55) A . . . for determining in a known manner whether hybridization has occurred. Such a probe analysis provides a rapid method for identifying "toxin"-encoding genes of the subject invention. Preferably, such genes would be cryIF genes whose core "toxin"-encoding portions can then be used with a crylA(b) or 436 protoxin-encoding portion to create a "chimeric" gene according to the subject invention. The nucleotide segments which are used as probes according to the invention can be. . .

(56) Certain "chimeric" toxins of the subject invention have been specifically exemplified herein. It should be readily apparent that the subject invention comprises. . . variant or equivalent toxins (and nucleotide sequences encoding equivalent toxins) having the same or similar pesticidal activity of the exemplified "toxin". Equivalent toxins will have amino acid homology with the exemplified "toxin". This amino acid homology will typically be greater than 75%, preferably be greater than 90%, and most preferably be greater than 95%. The amino acid homology will be highest in critical regions of the "toxin" which account for biological activity or are involved in the determination of three-dimensional configuration which ultimately is responsible for the. . .

(59) Recombinant hosts. A gene encoding a "chimeric" "toxin" of the subject invention can be introduced into a wide variety of microbial or plant hosts. Expression of the "toxin" gene results, directly or indirectly, in the intracellular production and maintenance of the pesticidal "chimeric" "toxin". With suitable microbial hosts, e.g., Pseudomonas, the microbes can be applied to the situs of the pest, where they will proliferate and be ingested. The result is control of the pest. Alternatively, the microbe hosting the "toxin" gene can be treated under conditions that prolong the activity of the "toxin" and stabilize the cell. The treated cell, which retains the toxic activity, then can be applied to the environment of. . .

(60) Where the gene encoding the "chimeric" "toxin" is introduced via a suitable vector into a microbial host, and said host is applied to the environment in a. . .

(62) A wide variety of ways are available for introducing a gene encoding a "chimeric" "toxin" into a microorganism host under conditions which allow for the stable maintenance and expression of the gene. These methods are. . .

(63) Treatment of cells. As mentioned above, recombinant cells producing the "chimeric" "toxin" of the subject invention can be treated to prolong the toxic activity and stabilize the cell. The pesticide microcapsule that is formed comprises the B.t. "toxin" within a cellular structure that has been stabilized and will protect the "toxin" when the microcapsule is applied to the environment of the target pest. Suitable host cells may include either prokaryotes or. . .

(65) Treatment of the microbial cell, e.g., a microbe containing the gene encoding a "chimeric" "toxin" of the subject invention, can be by chemical or physical means, or by a combination of chemical and/or physical means, so long as the technique does not deleteriously affect the properties of the "toxin", nor diminish the cellular capability of protecting the "toxin". Examples of chemical reagents are halogenating agents, particularly halogens of atomic no. 17-80. More particularly, iodine can be used under. . . and Company, 1967); or a combination of physical (heat) and chemical agents that preserve and prolong the activity of the "toxin" produced in the cell when the cell is administered to the host environment. Examples of physical means are short wavelength. . .

(68) Growth of cells. The cellular host containing the gene encoding a "chimeric" "toxin" of the subject invention may be grown in any convenient nutrient medium, where the DNA construct provides a selective advantage,. . .

(69) Formulations. Recombinant microbes comprising a gene encoding a "chimeric" "toxin" disclosed herein, can be formulated into bait granules and applied to the soil. Formulated product can also be applied as. . .

(85) A . . . be found in EPO patent application 0 471 564. A crylA(c)/crylA(b) gene, referred to herein as the 436 gene and "toxin", are described in U.S. Pat. No. 5,055,294. A plasmid designated pMYC1050 contains a crylA(c)/crylA(b) "chimeric" gene known as the 420 gene. pMYC1050 was constructed by re-cloning the "toxin" gene and promoter of pM3,130-7 (disclosed in U.S. Pat. No. 5,055,294) into a pTJS260-based vector such as pMYC467 (disclosed in U.S. Pat. No. 5,169,760) by methods well known in the art. In particular, the pM3,130-7 promoter and "toxin" gene can be obtained as a BamHI to NdeI fragment and placed into the pMYC467 plasmid replacing a fragment bounded. . .

(110) A "toxin"-containing DNA fragment was generated by PCR with primers L/D on template pMYC1260. The DNA was digested with BglII and PvuI. . . correct plasmids were identified by PCR analysis and agarose-TBE gel electrophoresis using the primer set N/O, which bridges the BamHI/BglII "fusion" junction. DETD(152) A second type of "chimeric" "toxin" was assembled by substituting the 436 protoxin module for the crylA(b) protoxin in pMYC2523 (FIG. 8). The 436 protoxin sequence. . .

(160) Insertion of the Gene Encoding the "Chimeric" "Toxin" Into Plants DETD(162) The gene encoding the "chimeric" "toxin", as disclosed herein, can be inserted into plant cells using a variety of techniques which are well known in the. . . higher plants. The vectors comprise, for example, pBR322, pUC series, M13mp series, pACYC184, etc. Accordingly, the sequence encoding the B.t. "toxin" can be inserted into the vector at a suitable restriction site. The resulting plasmid is used for transformation into E.. . .

(169) Cloning of the Gene Encoding the "Chimeric" "Toxin" Into Insect Viruses DETD(170) A. . . genome of the insect virus, thus enhancing the pathogenicity of the virus. Methods for constructing insect viruses which comprise the "chimeric" "toxin" gene are well known and readily practiced by those skilled in the art. These procedures are described, for example, in. .

We claim

1. An isolated polynucleotide molecule comprising a nucleotide sequence encoding a Bacillus "thuringiensis" "toxin" wherein said Bacillus "thuringiensis" "toxin" is a "chimeric" "toxin" comprising a cryIF core N-terminal "toxin" portion and a heterologous protoxin portion from a crylA(b) or a crylA(c)/crylA(b) "chimeric" "toxin".

CLMS(2) 2. The isolated polynucleotide molecule, according to claim 1, comprising a nucleotide sequence encoding a "chimeric" Bacillus "thuringiensis" "toxin" of approximately 1150 to 1200 amino acids, wherein said "toxin" comprises a cryIF core N-terminal sequence of at least about 590 amino acids and no more than about 1100 amino. . . acids, and wherein said crylA(b) or crylA(c)/crylA(b) protoxin portion comprises at least 100 amino acids at the C-terminus of said "toxin".

CLMS(15) 15. A substantially pure "chimeric" Bacillus "thuringiensis" "toxin" comprising a cryIF core N-terminal "toxin" portion and a heterologous C-terminal protoxin portion from a crylA(b) "toxin" or crylA(b)/crylA(c) "chimeric" "toxin".

CLMS(16) 16. The "chimeric" Bacillus "thuringiensis" "toxin", according to claim 15, having approximately 1150 to 1200 amino acids, wherein said "toxin" comprises a cryIF core N-terminal sequence of at least about 590 amino acids and no more than about 1100 amino acids, wherein said crylA(b) or crylA(c)/crylA(b) protoxin portion comprises at least 100 amino acids at the C-terminus of said "toxin".

CLMS(17) 17. The "chimeric" Bacillus "thuringiensis" "toxin", according to claim 16, wherein the transition from cryIF core N-terminal "toxin" portion to heterologous protoxin portion occurs after the sequence shown in SEQ ID NO. 30 and before the end of. . .

CLMS(18) 18. The "chimeric" Bacillus "thuringiensis" "toxin", according to claim 17, wherein said core "toxin" portion comprises the first about 601 amino acids of a cryIF "toxin" and wherein said C-terminal protoxin portion comprises the crylA(b) or crylA(c)/crylA(b) amino acid sequence which follows the peptide sequence shown. . .

CLMS(21) 21. The "chimeric" Bacillus "thuringiensis" "toxin", according to claim 15, comprises an amino acid sequence shown in FIG. 9.

US PAT NO: 5,516,693 [IMAGE AVAILABLE] L10: 18 of 31
ABSTRACT: The instant invention is drawn to plasmid p1BN10 harbored in E. coli DSM 4020 and coding for a Bacillus "thuringiensis" "endotoxin" (Bt8 "toxin") "fused" in frame to the neo gene of pBR322.

BSUM(36) Since kanamycin resistance is a most suitable selection marker both in bacteria and in Cyanobacteria, such gene "fusions" have promising applications. Indeed when using such NPTII "fusion" proteins to transform Cyanobacteria, a selection for kanamycin resistance allows direct selection for expression of the "fusion" product. Therefore, "toxin" gene "fusions" with neo may be used to transform Cyanobacteria and select for transformants expressing high levels of "toxin", by selection for kanamycin resistance. This selection procedure is particularly useful in a "shotgun" approach whereby the "fusion" gene is inserted randomly behin Cyanobacterium DNA sequences before transformation. This allows to directly select for those constructs comprising the "fusion" gene behind a strong promoter inducing high levels of the "fusion" protein in Cyanobacteria.

DETD(2) Cloning of the Bacillus "thuringiensis" subsp. israelensis (Bti) "toxin" gene

DETD(84) Cyanobacterium . . . enzymes. Southern blotting of the digested DNA showed that the 1.8 kb XbaI fragment from 5' end of the bt8 "toxin" gene, used as probe, hybridized with a 3.4 kb EcoRI and with a 3.6 kb BamHI fragment of the cyanobacterium chromosomal DNA. This result indicates that the bt8:neo "fusion" gene did integrate into the chromosome of cyanobacterium clones 20 and 43.

DETD(133) Thesis of Chanun Angsuthanasombat, Molecular cloning and expression of A .delta.-"endotoxin" gene of Bacillus "Thuringiensis" var. israelensis in Escherichia coli, Mahidol University, 1985, Bangkok--Thailand

US PAT NO: 5,508,264 [IMAGE AVAILABLE] L10: 19 of 31
ABSTRACT: Disclosed are compositions and processes for controlling lepidopteran pests. These compositions comprise synergistic combinations of a CryIF "chimeric" and CryIA(c) "chimeric" Bacillus "thuringiensis" .delta.-"endotoxin". These compositions have been found to exhibit excellent activity against lepidopteran pests.

BSUM(2) The soil microbe Bacillus "thuringiensis" (B.t) is a Gram-positive, spore-forming bacterium characterized by parasporal crystalline protein inclusions. These inclusions often appear microscopically as distinctively shaped crystals. The proteins can be highly toxic to pests and specific in their toxic activity. Certain B.t. "toxin" genes have been isolated and sequenced, and recombinant DNA-based B.t. products have been produced and approved for use. In addition,. . . approaches for delivering these B.t. endotoxins to agricultural environments are under development, including the use of plants genetically engineered with "endotoxin" genes for insect resistance and the use of stabilized intact microbial cells as B.t. "endotoxin" delivery vehicles (Gaertner, F. H., L. Kim [1988] TIBTECH 6:S4-S7). Thus, isolated B.t. "endotoxin" genes are becoming commercially valuable.

BSUM(3) Until . . . has been largely restricted to a narrow range of lepidopteran (caterpillar) pests. Preparations of the spores and crystals of B. "thuringiensis" subsp. kurstaki have been used for many years as commercial insecticides for lepidopteran pests. For example, B. "thuringiensis" var. kurstaki HD-1 produces a crystal called a .delta.-"endotoxin" which is toxic to the larvae of a number of lepidopteran insects.

BSUM(8) A majority of Bacillus "thuringiensis" .delta.-"endotoxin" crystal protein molecules are composed of two functional segments: The protease-resistant core "toxin" is the first segment and corresponds to about the first half of the protein molecule. The three-dimensional structure of a core segment of a cryIIIA B.t. .delta.-"endotoxin" is known and it is proposed that all related toxins have that same overall structure (Li, J., J. Carroll, D.. . . this second segment will be referred to herein as the "protoxin segment." The protoxin segment is believed to participate in "toxin" crystal formation (Arvidson, H., P. E. Dunn, S. Strand, A. I. Aronson [1989] Molecular Microbiology 3:1533-1534; Choma, C. T., W.. . . K. Surewicz, P. R. Carey, M. Pozsgay, T. Raynor, H Kaplan [1990] Eur. J. Biochem. 189:523-527). The full 130 kDa "toxin" molecule is rapidly processed to the resistant core segment by protease in the insect gut. The protoxin segment may thus convey a partial insect specificity for the "toxin" by limiting the accessibility of the core to the insect by reducing the protease processing of the "toxin" molecule (Haider, M. Z., B. H. Knowles, D. J. Ellar [1986] Eur. J. Biochem. 156:531-540) or by reducing "toxin" solubility (Aronson, A. I., E. S. Han, W. McGaughey, D. Johnson [1991] Appl. Environ. Microbiol. 57:981-986).

BSUM(9) "Chimeric" proteins joined within the "toxin" domains have been reported between CryIC and CryIA(b) (Honee, G., D. Convents, J. Van Rie, S. Jansens, M. Perferoen, B. Visser [1991] Mol. Microbiol. 5:2799-2806); however, the activity of these "chimeric" proteins was either much less, or undetectable, when compared to CryIC on a relevant insect.

BSUM(10) Honee et al. (Honee, G., W. Vriezen, B. Visser [1990] Appl. Environ. Microbiol. 56:823-825) also reported making a "chimeric" "fusion" protein by linking tandem "toxin" domains of CryIC and CryIA(b). The resulting protein had an increased spectrum of activity equivalent to the combined activities of the individual toxins; however, the activity of the "chimeric" was not increased toward any one of the target insects.

BSUM(14) The subject invention concerns the discovery of advantageous increased activity against lepidopteran pests achieved by the combination of two Bacillus "thuringiensis" (B.t.) delta-"endotoxin" proteins. More specifically, a CryIF "chimeric" "toxin" combined with a CryIA(c) "chimeric" "toxin" act in synergy to yield unexpected enhanced toxicity to lepidopteran pests.

BSUM(16) "Chimeric" CryIF genes useful according to the subject invention can be assembled that substitute a heterologous protoxin segment for all or. . . can be used in place of all or part of the region which encodes the protoxin for a native cryIF "toxin". Similarly, a "chimeric" gene can be constructed wherein the region encoding all or part of the protoxin of a cryIF "toxin" is replaced by DNA encoding all or part of the protoxin of a cryIA(c)/cryIA(b) "chimeric" gene. In a specific embodiment, the cryIA(c)/cryIA(b) "chimeric" gene is that which has been denoted 436 and which is described in U.S. Pat. No. 5,128,130. This gene can. . .

DRAWING DESC: DRWD(5) FIG. 4--The NsiI "toxin"-containing fragment with the new restriction sites is ligated to the vector-containing DNA from pMYC1050.DELTA.BamHI to give pMYC2244. ABamHI-PvuI PCR-derived DNA fragment containing the cryIF "toxin" is exchanged for the equivalent fragment in pMYC2244. The resulting "chimera" is called pMYC2239. B=BamHi, C=ClaI, H=HindIII, N=NsiI, P=PvuI.

DRWD(6) FIG. 5--The small ApaI DNA fragment of pMYC2047 is substituted for the homologous region of pMYC2239 to give plasmid pMYC2244. This "chimera" consists of cryIF in the "toxin" region and cryIA(b) in the protoxin. C=ClaI, H=HindIII, N=NsiI, P=PvuI.

DRWD(9) FIG. 8--A "chimeric" "toxin" containing the 436 protoxin is constructed by substituting a PCR-generated PvuI-BstEII protoxin DNA for the homologous fragment in pMYC2523. The. . .

DETD(23) SEQ ID NO. 22 shows the "toxin"-encoding DNA sequence of pMYC2244, which encodes a cryIF/cryIA(b) "chimeric" "toxin".

DETD(24)
SEQ ID NO. 23 shows the predicted amino acid sequence of the cryIF/cryIA(b) "chimeric" "toxin" encoded by pMYC2244.

DETD(27) SEQ ID NO. 26 shows the "toxin"-encoding DNA sequence of pMYC2523, which encodes a cryIF/cryIA(b) "chimeric" "toxin" with codon rework.

DETD(29) SEQ ID NO. 28 shows the "toxin"-encoding DNA sequence of pMYC2254, which encodes a cryIF/436 "chimeric" "toxin".

DETD(37) The subject invention concerns the unexpected enhanced pesticidal activity resulting from the combination of a CryIF "chimeric" "toxin" and a CryIA(c) "chimeric" "toxin". The combination surprisingly has increased activity against lepidopteran pests. Preparations of combinations of isolates that produce the two "chimeric" toxins can be used to practice the subject invention. Pseudomonas fluorescens cells transformed with B.t. genes can serve as one. . . of the toxins of the subject invention. For example, a lactose-inducible P. fluorescens strain comprising a gene encoding a CryIF/CryIA(b) "toxin", and P. fluorescens MR436, which comprises a gene encoding a CryIA(c)/CryIA(b) "chimeric" "toxin", can be used to practice the subject invention. These two Pseudomonas strains can be combined in a physical blend that. . .

DETD(41) In accordance with the subject invention, it has been discovered that products comprising the two "chimeric" toxins have been discovered to require a lower total protein content for product application, thus providing the user greater economy. Insects which are less susceptible to the action of a single "toxin" will be more greatly affected by the combination of toxins of the subject invention, rendering a product containing the two toxins more efficacious than products containing a single "toxin". Additionally, pests are less likely to develop a rapid resistance to a product containing the two toxins, than to products containing a single "toxin".

DETD(43) The "chimeric" toxins of the subject invention comprise a full core N-terminal "toxin" portion of a B.t. "toxin" and, at some point past the end of the "toxin" portion, the protein has a transition to a heterologous protoxin sequence. The N-terminal "toxin" portion of a B.t. "toxin" is referered to herein as the "core" "toxin". The transition to the heterologous protoxin segment can occur at approximately the "toxin"/protoxin junction or, in the alternative, a portion of the native protoxin (extending past the "toxin" portion) can be retained with the transition to the heterologous protoxin occurring downstream. As an example, one "chimeric" "toxin" of the subject invention has the full "toxin" portion of cryIF (amino acids 1-601) and a heterologous protoxin (amino acids 602 to the C-terminus). In a preferred embodiment, the heterologous portion of the protoxin is derived from a cryIA(b) or 436 "toxin".

DETD(44) A. . . certain class such as cryIF, will vary to some extent in length and the precise location of the transition from "toxin" portion to protoxin portion. Typically, the cryIA(b) and cryIF toxins are about 1150 to about 1200 amino acids in length. The transition from "toxin" portion to protoxin portion will typically occur at between about 50% to about 60% of the full length "toxin". The "chimeric" "toxin" of the subject invention will include the full expanse of this core N-terminal "toxin" portion. Thus, the "chimeric" "toxin" will comprise at least about 50% of the full length cryIF B.t. "toxin". This will typically be at least about 590 amino acids. With regard to the protoxin portion, the full expanse of the cryIA(b) protoxin portion extends from the end of the "toxin" portion to the C-terminus of the molecule. It is the last about 100 to 150 amino acids of this portion which are most critical to include in the "chimeric" "toxin" of the subject invention. In a "chimeric" "toxin" specifically exemplified herein, at least amino acids 1043 (of SEQ ID NO. 23) to the C-terminus of the cryIA(b) molecule. . . marks the location in the protoxin segment of the molecule beyond which heterologous amino acids will always occur in the "chimeric" "toxin". In another example, the peptide shown as SEQ ID NO. 31 occurs at amino acids 1061 to 1068. In this. . . approximately 5 to 10% of the overall B.t. protein which should comprise heterologous DNA (compared to the cryIF core N-terminal "toxin" portion) in the "chimeric" "toxin" of the subject invention. In the specific examples contained herein, heterologous protoxin sequences occur from amino acid 640 to the. . .

DETD(45) Thus, a preferred embodiment of the subject invention is a "chimeric" B.t "toxin" of about 1150 to about 1200 amino acids in length, wherein the "chimeric" "toxin" comprises a cryIF core N-terminal "toxin" portion of at least about 50 to 60% of a full cryIF molecule, but no more than about 90 to 95% of the full molecule. The "chimeric" "toxin" further comprises a cryIA(b) or a 436 protoxin C-terminal portion which comprises at least about 5 to 10% of the. . . transition from cryIF to cryIA(b) or 436 sequence thus occurs within the protoxin segment (or at the junction of the "toxin" and protoxin segments) between about 50% and about 95% of the way through the molecule. In the specific examples provided. . .

DETD(46) A specific embodiment of the subject invention is the "chimeric" "toxin" shown in FIG. 9. Other constructs may be made and used by those skilled in this art having the benefit of the teachings provided herein. The core "toxin" segment of cryI proteins characteristically ends with the sequence: Val/Leu Tyr/Ile Ile Asp Arg/Lys Ile/Phe Glu Ile/Phe/Leu Ile/Leu/Val Pro/Leu Ala/Val. . . NO. 23. Additionally, the protoxin segments of the cryI toxins (which follow residue 601) bear more sequence similarity than the "toxin" segments. Because of this sequence similarity, the transition point in the protoxin segment for making a "chimeric" protein between the cryIF sequence and the cryIA(b) or 436 sequence can be readily determined by one skilled in the. . .

DETD(47) Therefore a "chimeric" "toxin" of the subject invention can comprise the full cryIF "toxin" and a portion of the cryIF protoxin, transitioning to the corresponding cryIA(b) or 436 sequence at any position between the end of the "toxin" segment (as defined above) and the end of the peptide sequence shown in SEQ ID NO. 31. Preferably, the amino acid sequence of the C-terminus of the "chimeric" "toxin" comprises a cryIA(b) sequence or a sequence from the 436 gene or an equivalent of one of these sequences.

DETD(51) The. . . can be carried out according to the subject invention. BamHI and PvuI cloning sites can be introduced into a cryIA(c)/cryIA(b) "chimeric" "toxin" gene by mutagenesis using the PCR technique of Splice Overlap Extension (SOE) (Horton, R. M., H. D. Hunt, S. N.. . . pMYC2224. The new plasmid, which we designated pMYC2239, consisted of a short segment of cryIA(c) followed by cryIF to the "toxin"/protoxin segment junction. Thus, the protoxin segment was now derived from cryIA(b) (pMYC1050). An ApaI fragment derived from the cryIF clone. . . substituted for the ApaI fragment in pMYC2239. The resulting clone (pMYC2244) consisted of cryIF from the initiator methionine to the "toxin"/protoxin segment junction and cryIA(b) to the end of the coding region. Clone pMYC2243 was constructed by SOE to introduce silent. . . from pMYC2243 that contained the silent changes was substituted for the ApaI fragment in pMYC2244 to give clone pMYC2523. The "chimeric" pMYC2523 showed an expression improvement over pMYC2243, which contains unchanged cryIF protein sequence.

DETD(64) Treatment of cells. Bacillus "thuringiensis" or recombinant cells expressing the B.t. toxins can be treated to prolong the "toxin" activity and stabilize the cell. The pesticide microcapsule that is formed comprises the B.t. "toxin" or toxins within a cellular structure that has been stabilized and will protect the "toxin" when the microcapsule is applied to the environment of the target pest. Suitable host cells may include either prokaryotes or. . .

DETD(113) A "toxin"-containing DNA fragment was generated by PCR with primers L/D on template pMYC1260. The DNA was digested with BglII and PvuI. . . correct plasmids were identified by PCR analysis and agarose-TBE gel electrophoresis using the primer set N/O, which bridges the BamHI/BglII "fusion" junction.

DETD(155) A second type of "chimeric" "toxin" was assembled by substituting the 436 protoxin module for the cryIA(b) protoxin in pMYC2523 (FIG. 8). The 436 protoxin sequence. . .

DETD(163) Analysis for Synergy Between CryIF "Chimeric" "Toxin" and CryIA(c) "Chimeric" "Toxin" Against the Corn Earworm, Heliothis zea

DETD(174) TABLE 2

% INHIBITION					
	cryIF/	cryIA(c)/	1:1 mix of the two		
Rate	cryIA(b)	cryIA(b)	"chimeric" toxins		
.mu.g "toxin"/g diet					
	a	b	E(exp)	E(obs) SF	
50.0	–	–	50	78	1.6
25.0	13	23	22	62. . .	

DETD(176) Analysis for Synergy Between CryIF "Chimeric" "Toxin" and CryIA(c) "Chimeric" "Toxin" Against the Corn Earworm, Heliothis zea

We claim:
1. A composition for controlling lepidopteran pests comprising a CryIF "chimeric" core "toxin"-containing protein and a CryIA(c) "chimeric" core "toxin"-containing protein.

CLMS(2) 2. The composition, according to claim 1, wherein said CryIF "chimeric" core "toxin"-containing protein comprises a CryIF core N-terminal protein portion and a heterologous C-terminal "toxin" portion from a CryIA(b) "toxin" or CryIA(b)/CryIA(c) "chimeric" "toxin".

CLMS(3) 3. The composition, according to claim 2, wherein said CryIF "chimeric" core "toxin"-containing protein has approximately 1150 to 1200 amino acids and comprises a CryIF core N-terminal sequence of at least about 590. . .

CLMS(8) 8. The composition, according to claim 1, wherein said CryIA(c) "chimeric" core "toxin"-containing protein has an amino acid sequence comprising the sequence shown in SEQ ID NO. 34.

CLMS(9) 9. . . . comprising contacting said pests, or the environment of said pests, with an effective amount of a composition comprising a CryIF "chimeric" core "toxin"-containing protein and a CryIA(c) "chimeric" core "toxin"-containing protein.

CLMS(10) 10. The method, according to claim 9, wherein said CryIF "chimeric" core "toxin"-containing protein comprises a CryIF core N-terminal "toxin" portion and a heterologous C-terminal protoxin portion from a CryIA(b) "toxin" or CryIA(b)/CryIA(c) "chimeric" "toxin".

CLMS(11) 11. The method, according to claim 10, wherein said CryIF "chimeric" core "toxin"-containing protein has approximately 1150 to 1200 amino acids and comprises a CryIF core N-terminal sequence of at least about 590. . .

CLMS(16) 16. The method, according to claim 10, wherein said CryIA(c) "chimeric" core "toxin"-containing protein has an amino acid sequence comprising the sequence shown in SEQ ID NO. 34.

CLMS(17) 17. The method, according to claim 10, wherein said CryIF "chimeric" and CryIA(c) "chimeric" core "toxin"-containing proteins are from a host cell transformed to express SEQ ID NO. 23 and SEQ ID NO. 34.

US PAT NO: 5,500,365 [IMAGE AVAILABLE] L10: 20 of 31

BSUM(22) Therefore, . . . genes. It is yet another object of the present invention to provide synthetic plant genes which express the crystal protein "toxin" of Bacillus "thuringiensis" at relatively high levels.

DETD(2) The. . . present invention will be primarily described with respect to the preparation of synthetic plant genes which encode the crystal protein "toxin" of Bacillus "thuringiensis" (B.t.). Suitable B.t. subspecies include, but are not limited to, B.t. kurstaki HD-1, B.t. kurstaki HD-73, B.t. sotto, B.t. berliner, B.t. "thuringiensis", B.t. tolworthi, B.t. dendrolimus, B.t. alesti, B.t. galleriae, B.t. aizawai, B.t. subtoxicus, B.t. entomocidus, B.t. tenebrionis and B.t. san diego. . . the present method may be used to prepare synthetic plant genes which encode nonplant proteins other than the crystal protein "toxin" of B.t. as well as plant proteins (see for instance, Example 9).

DETD(5) It. . . improper expression of the gene. It was suggested that this truncated mRNA was too sho[illegible] encode a functional truncated "toxin", but there must have been a low level of longer mRNA in some plants o[illegible] insecticidal activity would have. . . (Barton et al., 1987). The above illustrates that lepidopteran type B.t. genes are poorly expressed in plants compared to other "chimeric" genes previously expressed from the same promoter cassettes.

DETD(31) The. . . of the present invention the enhancement method has been applied to design modified and fully synthetic genes encoding the crystal "toxin" protein of Bacillus "thuringiensis". The structural genes of the present invention may optionally encode a "fusion" protein comprising an amino-terminal chloroplast transit peptide or secretory signal sequence (see for instance, Examples 10 and 11).

DETD(69) The crystal protein "toxin" from B.t.k. HD-73 exhibits a higher unit activity against some important agricultural pests. The "toxin" protein of HD-1 and HD73 exhibit substantial homology (.about.90%) in the N-terminal 450 amino acids, but differ substantially in the amino acid region 451-615. "Fusion" proteins comprising amino acids 1-450 of HD-1 and 451-615 of HD73 exhibit the insecticidal properties of the wild-type HD-73. The. . .

We claim:
1. A modified "chimeric" gene comprising a promoter which functions in plant cells operabiy linked to a structural coding sequence and a 3' non-translated. . . cause the addition of polyadenylate nucleotides to the 3' end of the RNA, wherein said structural coding sequence encodes a "toxin" protein derived from a Bacillus "thuringiensis" protein, wherein said structural coding sequence comprises a DNA sequence which differs from the naturally occurring DNA sequence encoding said Bacillus "thuringiensis" protein and comprises the following characteristics: said naturally occurring DNA sequence comprises a region having the following sequence; ##STR1## and. . .

CLMS(2) 2. The modified "chimeric" gene of claim 1 wherein said modifications increase the number of plant preferred codons in said structural coding sequence.

CLMS(3) 3. The modified "chimeric" gene of claim 1 wherein said Bacillus "thuringiensis" is Bacillus "thuringiensis" var. kurstaki.

CLMS(4) 4. A modified "chimeric" gene comprising a promoter which functions in plant cells operably linked to a structural coding sequence and a 3' non-translated. . . cause the addition of polyadenylate nucleotides to the 3' end of the RNA, wherein said structural coding sequence encodes a "toxin" protein derived from a Bacillus "thuringiensis" protein, wherein said structural coding sequence comprises a DNA sequence which differs from the naturally occurring DNA sequence encoding said Bacillus "thuringiensis" protein and comprises the following characteristics: said naturally occurring DNA sequence comprises a region having the following sequence: ##STR2## and. . .

CLMS(5) 5. The modified "chimeric" gene of claim 4 wherein said modifications increase the number of plant preferred codons in said structural coding sequence.

CLMS(6) 6. The modified "chimeric" gene of claim 4 wherein said Bacillus "thuringiensis" is Bacillus "thuringiensis" var. kurstaki.

CLMS(7) 7. A modified "chimeric" gene comprising a promoter which functions in plant cells operably linked to a structural coding sequence and a 3' non-translated. . . cause the addition of polyadenylate nucleotides to the 3' end of the RNA, wherein said structural coding sequence encodes a "toxin" protein derived from a Bacillus thuringienis protein, wherein said structural coding sequence comprises a DNA sequence which differs from the naturally occurring DNA sequence encoding said Bacillus "thuringiensis" protein and comprises the following characteristics: said naturally occurring DNA sequence comprises a region having the following sequence: ##STR3## and. . .

CLMS(8) 8. The modified "chimeric" gene of claim 7 wherein said modifications increase the number of plant preferred codons in said structural coding sequence.

CLMS(9) 9. The modified "chimeric" gene of claim 7 wherein said Bacillus "thuringiensis" is Bacillus "thuringiensis" var. kurstaki.

CLMS(10) 10. A transformed plant cell comprising a modified "chimeric" gene which comprises a promoter which functions in plant cells operably linked to a structural coding sequence and a 3'. . . cause the addition of polyadenylate nucleotides to the 3' end of the RNA, wherein said structural coding sequence encodes a "toxin" protein derived from a Bacillus "thuringiensis" protein, wherein said structural coding sequence comprises a DNA sequence which differs from the naturally occurring DNA sequence encoding said Bacillus "thuringiensis" protein and has characteristics comprising the following: said naturally occurring DNA sequence comprises a region having the following sequence: ##STR4##. . .

CLMS(11) 11. A transformed plant cell comprising a modified "chimeric" gene which comprises a promoter which functions in plant cells operably linked to a structural coding sequence and a 3'. . . cause the addition of polyadenylate nucleotides to the 3' end of the RNA, wherein said structural coding sequence encodes a "toxin" protein derived from a Bacillus "thuringiensis" protein, wherein said structural coding sequence comprises a DNA sequence which differs from the naturally occurring DNA sequence encoding said Bacillus "thuringiensis" protein and has characteristics comprising the following: said naturally occurring DNA sequence comprises a region having the following sequence: ##STR5##. . .

CLMS(12) 12. A transformed plant cell comprising a modified "chimeric" gene which comprises a promoter which functions in plant cells operably linked to a structural coding sequence and a 3'. . . cause the addition of polyadenylate nucleotides to the 3' end of the RNA, wherein said structural coding sequence encodes a "toxin" protein derived from a Bacillus "thuringiensis" protein, wherein said structural coding sequence comprises a DNA sequence which differs from the naturally occurring DNA sequence encoding said Bacillus "thuringiensis" protein and has characteristics comprising the following: said naturally occurring DNA sequence comprises a region having the following sequence: ##STR6##. . .

US PAT NO: 5,495,071 [IMAGE AVAILABLE] L10: 21 of 31

ABSTRACT: A method for producing genetically transformed plants exhibiting toxicity to Coleopteran insects is disclosed. In another aspect, the present invention embraces "chimeric" plant genes, genetically transformed cells and differentiated plants which exhibit toxicity to Coleopteran insects. In yet another aspect, the present invention embraces bacterial cells and plant transformation vectors comprising a "chimeric" plant gene encoding a Coleopteran "toxin" protein of Bacillus "thuringiensis".

BSUM(2) Bacillus "thuringiensis" (B.t.) is a spore forming soil bacterium which is known for its ability to produce a parasporal crystal protein which. . . butterflies) and a few are reported to have activity against Dipteran insects (mosquitoes and flies, see Aronson et al. 1986). "Toxin" genes from a variety of these strains have been cloned and the toxins have been expressed in heterologous hosts (Schnepf. . . var. san diego (B.t. sd., Herrnstadt et al., 1986) strains have been identified as having activity against Coleopteran insects. The "toxin" gene from B.t. sd. has been cloned, but the "toxin" produced in E. coli was reported to be a larger size than the "toxin" from B.t. sd. crystals, and activity of this recombinant B.t. sd. "toxin" was implied to be weak.

BSUM(3) Insects susceptible to the action of the protein "toxin" of Coleopteran-type Bacillus "thuringiensis" bacteria include, but are not limited to, Colorado potato beetle (Leptinotarsa decemlineata), boll weevil (Anthonomus grandis), yellow mealworm (Tenebrio molitor),. . .

BSUM(5) Although certain "chimeric" genes have been expressed in transformed plant cells and plants, such expression is by no means straight forward. Specifically, the expression of Lepidopteran-type B.t. "toxin" proteins has been particularly problematic. It has now been found that the teachings of the art with respect to expression of Lepidopteran-type B.t. "toxin" protein in plants do not extend to Coleopteran-type B.t. "toxin" protein. These findings are directly contrary to the prior teachings which suggested that one would employ the same genetic manipulations. . .

BSUM(9) ii) a DNA sequence that causes the production of a RNA sequence encoding a Coleopteran-type "toxin" protein of Bacillus "thuringiensis"; and

BSUM(15) (b) a DNA sequence that causes the production of a RNA sequence encoding a Coleopteran-type "toxin" protein of Bacillus "thuringiensis"; and

DETD(2) The . . . plants to exhibit toxicity toward susceptible Coleopteran insects. More particularly, the present invention provides transgenic plants which express the Coleopteran-type "toxin" protein of Bacillus "thuringiensis" at an insecticidal level.

DETD(8) The "chimeric" gene also contains a structural coding sequence which encodes the Coleopteran-type "toxin" protein of Bacillus "thuringiensis" or an insecticidally-active fragment thereof. Exemplary sources of such structural coding sequences are B.t. tenebronis and B. t. san diego. Accordingly, in exemplary embodiments the present invention provides a structural coding sequence from Bacillus "thuringiensis" var. tenebrionis and insecticidally-active fragments thereof. Those skilled in the art will recognize that other structural coding sequence substantially homologous to the "toxin" coding sequence of B.t.t. can be utilized following the teachings described herein and are, therefore, within the scope of this. . .

DETD(11) The plant material thus modified can be assayed, for example, by Northern blotting, for the presence of Coleopteran-type "toxin" protein mRNA. If no "toxin" protein mRNA (or too low a titer) is detected, the promoter used in the "chimeric" gene construct is replaced with another, potentially stronger promoter and the altered construct retested. Alternately, level of "toxin" protein may be assayed by immunoassay such as Western blot. In many cases the most sensitive assay for "toxin" protein is insect bioassay.

DETD(32) Using . . . sequence information, synthetic DNA probes (FIG. 1) were designed which were used in the isolation of clones containing the B.t.t. "toxin" gene. Probes were end-labeled with [.gamma.-.sup.32 P] ATP according to Maniatis (1982). B. "thuringiensis" var. tenebrionis was grown for 6 hours at 37.degree. C. in Spizizen medium (Spizizen, 1958) supplemented with 0.1% yeast extract. . .

DETD(67) Although the Coleopteran-type toxins and the Lepidopteran-type toxins are derived from Bacillus "thuringiensis", there are significant differences between the "toxin" genes and the "toxin" proteins of the two types. As isolated from Bacillus "thuringiensis" both types of toxins are found in parasporal crystals; however, as described above, the solubility properties of the crystals are distinctly different. In addition, the sizes of the "toxin" proteins found in solubilized crystals are completely different. Lepidopteran-type "toxin" proteins are typically on the order of 130 kDa while the Coleopteran-type "toxin" proteins are approximately 70 kDa.

DETD(149) "CHIMERIC". B.t.t. "TOXIN" GENE USING A MAS PROMOTER

DETD(153) "Chimeric" B.t.t. "toxin" genes driven by the MAS promoter are prepared by digesting either pMON9791 or pMON9792 with BglII, recovering the "toxin" encoding fragment and moving this fragment into pMON9741 following the teachings provided herein.

DETD(165) Shoot . . . streaked on an LB agar plate and grown for 2 to 3 days. pMON9753-ASE which is described above contains the "chimeric" B.t.t. "toxin" gene driven by the CaMV35S promoter. Alternatively, Agrobacterium strains pMON9791-ACO or pMON9792-ACO containing "chimeric" B.t.t. "toxin" genes are used. Stem sections are placed on 0.8% agar-solidified medium containing salts and organic addenda as in Jarret et. . potato cells are transformed. Uninoculated control tissue is inhibited at this concentration of kanamycin. Transformed potato tissue expresses the B.t.t. "toxin" gene. B.t.t. "toxin" mRNA may be detected by Northern analysis and B.t.t. "toxin" protein may be detected by immunoassay such as Western blot analysis. However, in many cases the most sensitive assay for the presence of B.t.t. "toxin" is the insect bioassay. Colorado potato beetle larvae feeding on the transformed tissue suffer from the effects of the "toxin".

DETD(171) When the Agrobacterium strain used for transformation contains a "chimeric" B.t.t. "toxin" gene such as pMON9753, pMON9791 or pMON9792, the B.t.t. "toxin" gene is expressed in the transformed callus, embryos derived from this callus, and in the transformed plants derived from the embryos. For all of these cases, expression of the B.t.t. "toxin" mRNA may be detected by Northern analysis, and expression of the B.t.t. "toxin" protein may be detected by immunoassay such as Western blot analysis. Insect bioassay may be the most sensitive measure for the presence of "toxin" protein.

DETD(174) The following description outlines the preparation of protoplasts from maize, the introduction of "chimeric" B.t.t. "toxin" genes into the protoplast by electroporation, and the recovery of stably transformed, kanamycin resistant maize cells expressing "chimeric" B.t.t. "toxin" genes.

DETD(180) As . . . al. (1986), transformed maize cells can be selected by growth in kanamycin containing medium following electroporation with DNA vectors containing "chimeric" kanamycin resistance genes composed of the CaMV35S promoter, the NPTII coding region and the NOS 3' end. pMON9791 and pMON9792 contain such "chimeric" NPTII genes and also contain "chimeric" B.t.t. "toxin" genes. As decribed above, maize protoplasts are transformed by electroporation with DNA vectors where the DNA vectors are pMON9791 or pMON9792. Following selection for kanamycin resistance, the transformed maize cells are assayed for expression of the B.t.t. "toxin" gene. Assays are performed for B.t.t. mRNA by Northern blot analysis and for B.t.t. "toxin" protein by immunoassay such as Western blot analysis.

We claim:

1. A "chimeric" gene capable of expressing in a plant cell comprising in sequence: (a) a promoter which functions in plants to cause the production of RNA; (b) a DNA sequence that causes the production of a RNA sequence encoding Coleopteran-type "toxin" protein of Bacillus "thuringiensis" var. tenebrionis having the amino acid sequence selected from the group consisting of from residues (1-644), residues (16-644), residues (48-644),. . .

CLMS(3) 3. A gene of claim 1 in which the DNA sequence encoding a Coleopteran-type "toxin" protein is from Bacillus "thuringiensis" var. tenebrionis.

CLMS(9) 9. The gene of claim 1 encoding the "toxin" protein of Bacillus "thuringiensis" var. tenebrionis having the amino acid sequence from residues (1-644) of said protein wherein the amino acid residues of said. . .

CLMS(10) 10. The gene of claim 1 encoding the "toxin" protein of Bacillus "thuringiensis" var. tenebrionis having the amino acid sequence from residues (16-644) of said protein wherein the amino acid residues of said. . .

CLMS(11) 11. A "chimeric" gene capable of expressing in a plant cell comprising in sequence: (a) a promoter which functions in plants to cause the production of RNA; (b) a DNA sequence that causes the production of a RNA sequence encoding Coleopteran-type "toxin" protein of Bacillus "thuringiensis" var. tenebrionis having the amino acid sequence from residues (48-644) of said protein wherein the amino acid residues of said. . .

CLMS(12) 12. The gene of claim 1 encoding the "toxin" protein of Bacillus "thuringiensis" var, tenebrionis having the amino acid sequence from residues (50-644) of said protein wherein the amino acid residues of said. . .

CLMS(13) 13. The gene of claim 1 encoding the "toxin" protein of Bacillus "thuringiensis" var. tenebrionis having the amino acid sequence from residues (58-644) of said protein wherein the amino acid residues of said. . .

CLMS(14) 14. The gene of claim 1 encoding the "toxin" protein of Bacillus "thuringiensis" var. tenebrionis having the amino acid sequence from residues (77-644) of said protein wherein the amino acid residues of said. . .

CLMS(15) 15. A DNA sequence that encodes a Coleopteran-type "toxin" protein of Bacillus "thuringiensis" var. tenebrionis which is effective in controlling Coleopteran-type insects having the amino acid sequence selected from the group consisting of. . .

CLMS(16) 16. A DNA sequence that encodes a Coleopteran-type "toxin" protein of Bacillus "thuringiensis" var. tenebrionis which is effective in controlling Coleopteran-type insects having the amino acid sequence from residues (48-644) of said protein. . .

CLMS(17) 17. A transformed plant cell expressing the "toxin" protein of Bacillus "thuringiensis" var. tenebrionis having the amino acid sequence from residues (48-644) of the full-length protein wherein the amino acid residues of. . .

CLMS(18) 18. A transformed plant selected from the group consisting of tomato and potato expressing the "toxin" protein of Bacillus "thuringiensis" var. tenebrionis having the amino acid sequence from residues (48-644) of the full-length protein wherein the amino acid residues of. . .

CLMS(19) 19. A substantially pure "toxin" protein of Bacillus "thuringiensis" var. tenebrionis having the amino acid sequence from residues (48-644) of the full-length protein wherein the amino acid residues of. . .

CLMS(20) 20. A "toxin" protein of Bacillus "thuringiensis" var. tenebrionis free of other proteins of Bacillus "thuringiensis" var. tenebrionis said "toxin" protein having the amino acid sequence from residues (48-644) of the full-length protein wherein the amino acid residues of said. . .

US PAT NO: 5,466,785 [IMAGE AVAILABLE] L10: 22 of 31

DETD(11) The . . . or stems, and not in the seed. Such proteins include, for example, insect selective toxins such as polypeptides from Bacillus "thuringiensis", which are postulated to generate small pores in the insect gut cell membrane, Knowles et al., Biochim. Biophys. Acta 924:509-518. . . Biochim. Biophys. Acta 939:57-63 (1988); sodium channel proteins and synthetic fragments, Oiki et al. PNAS USA 85:2393-2397 (1988); the alpha "toxin" of Staphylococcus aureus, Tobkes et al. Biochem. 24:1915-1920 (1985); apolipoproteins and fragments thereof, Knott et al., Science 230:37 (1985); Nakagawa. . .

CLMS(2) 2. A "chimeric" gene comprising: a) the promoter sequence located at nucleotide positions 1 to 2564 of the gene set forth in SEQ. . .

CLMS(3) 3. The "chimeric" gene of claim 2 wherein said coding sequence of interest encodes a Bacillus "thuringiensis" insect "toxin".

CLMS(4) 4. A recombinant DNA vector comprising the "chimeric" gene of claim 2.

CLMS(5) 5. A recombinant DNA vector comprising the "chimeric" gene of claim 3.

US PAT NO: 5,350,689 [IMAGE AVAILABLE] L10: 23 of 31

ABSTRACT: Methods . . . derived from embryogenic cell cultures or callus cultures. The protoplasts, cells and resulting plants may be transgenic, containing, for example, "chimeric" genes coding for a polypeptide having substantially the insect toxicity properties of the crystal protein produced by Bacillus "thuringiensis".

BSUM(12) Bacillus "thuringiensis" (hereinafter Bt) is a species of bacteria that produces a crystal protein, also referred to as delta-"endotoxin". This crystal protein is, technically, a protoxin that is converted into a "toxin" upon being ingested by larvae of lepidopteran, coleopteran and dipteran insects.

DRAWING DESC: DRWD(23) FIG. 13 shows the nucleotide sequence of the "endotoxin" gene from Bacillus "thuringiensis" vat. kurstaki HD1. A preferred sequence of nucleotides that codes for a crystal protein is that shown as nucleotides 156. . .

DETD(169) Accordingly, the polypeptide coded for by the chimetic gene of the present invention is preferably structurally related to the delta-"endotoxin" of the crystal protein produced by Bt. Bt produces a crystal protein with a subunit which is a protoxin having. . . have the requisite insecticidal activity. The protoxin, insecticidal fragments of the protoxin and insecticidal portions of these fragments can be "fused" to other molecules such as polypeptides .

DETD(193) In addition to the "chimeric" gene coding for a Bt "toxin" or a Bt-like "toxin", the vectors preferably further comprise a DNA sequence that permits the selection or screening of corn plant cells containing the

DETD(205) The . . . larvae comprising feeding the larvae an insecticidal amount of transgenic Zea mays cells containing a gene coding for a Bacillus "thuringiensis" crystal "toxin" or a polypeptide having substantially the insect toxicity properties of a Bacillus "thuringiensis" crystal protein.

DETD(256) Example 6a: Construction of pTOX, Containing a "Chimeric" Gene Encoding the Insecticidal "Toxin" Gene of Bacillus "thuringiensis" var tenebrionis

DETD(257) A gene encoding the insecticidal crystal protein gene of Bacillus "thuringiensis" var. tenebrionis has been characterized and sequenced [Sekar, V. et al., Proc. Natl. Acad Sci USA, 84 (1987) 7036-7040]. This. . . the 35S RNA transcript of CaMV [cauliflower mosaic virus] separated by a unique BamH1 site. The restriction fragment bearing the "toxin" coding sequence is made compatible to the unique BamHI site of pCIB 770 by use of the appropriate molecular adapter. . .

DETD(258) Example 6b: Construction of pSAN, Containing a Chimetic Gene Encoding the Insecticidal "Toxin" Gene of Bacillus "thuringiensis" strain san diego

DETD(259) A gene encoding the insecticidal protein of Bacillus "thuringiensis" strain san diego has been characterized and sequenced by Herrnstadt et al., EP-0-202-739 and EP-0-213-818. This coding sequence is isolated. . . the 35S RNA transcript of CaMV [cauliflower mosaic virus] separated by a unique BamH site. The restriction fragment bearing the "toxin" coding sequence is made compatible to the unique BamHI site of pCIB 770 by use of the appropriate molecular adapter. . .

US PAT NO: 5,317,096 [IMAGE AVAILABLE] L10: 24 of 31

TITLE: Transformation vectors allowing expression of foreign polypeptide endotoxins from Bacillus "thuringiensis" in plants

ABSTRACT: Novel transformation vectors containing novel "chimeric" genes allow the introduction of exogenous DNA fragments coding for polypeptide toxins produced by Bacillus "thuringiensis" or having substantial sequence homology to a gene coding for a polypeptide "toxin" as described herein and expression of the "chimeric" gene in plant cells and their progeny after integration into the plant cell genome. Transformed plant cells and their progeny exhibit stably inherited polypeptide "toxin" expression useful for protecting said plant cells and their progeny against certain insect pests and in controlling said insect pests.

BSUM(2) This . . . the use of genetic engineering techniques in the modification of plants. More particularly, it concerns introduction and integration of a "chimeric" gene coding for a polypeptide "toxin" produced by Bacillus "thuringiensis" or having substantial sequence homology to a "toxin" gene described below in plant cells and obtaining an insect controlling level of expression of said polypeptide "toxin" intracellularly by transformed plant cells and their progeny.

BSUM(7) Bacillus "thuringiensis" (referred to at times herein as B.t.) bacteria includes approximately 19 known varieties that produce polypeptide toxins which form parasporal. . . by insect larvae, the crystals are solubilized and processed in the insect midgut to yield at least one active polypeptide "toxin" which is believed to act on the midgut cell membrane. Studies have revealed that individual crystal polypeptides exhibit insecticidal activity.. . .

BSUM(12) It is one object of this invention to provide novel "chimeric" genes coding for the polypeptide "toxin" produced by Bacillus "thuringiensis", or coding for a polypeptide "toxin" having substantial sequence homology to a "toxin" gene described herein. The "chimeric" genes' plant regulatory sequences direct expression in transformed plant cells.

BSUM(19) (b) at least one DNA fragment coding for a polypeptide "toxin" produced by Bacillus "thuringiensis" or having substantial sequence homology thereto.

BSUM(25) (ii) at least one DNA fragment coding for a polypeptide "toxin" produced by Bacillus "thuringiensis" or at least one DNA fragment having substantial sequence homology thereto.

BSUM(30) (b) at least one DNA fragment coding for a polypeptide "toxin" produced by Bacillus "thuringiensis", or at least one DNA fragment having substantial sequence homology thereto.

BSUM(34) Transformed plant cells and their progeny intracellularly express a polypeptide "toxin" substantially similar to the polypeptide toxins produced by Bacillus "thuringiensis" and are substantially toxic to certain insects. Transformed plant cells and their progeny may be used in controlling said insects.

DETD(7) (1) isolation of at least one DNA fragment from Bacillus "thuringiensis" coding for a polypeptide "toxin" by digestion of bacterial DNA and inserting the mixture of DNA fragments obtained into a cloning vehicle harbored in a. . .

DETD(25) Transformed plant cells and their progeny should express a polypeptide "toxin" substantially similar to polypeptide toxins being produced by Bacillus "thuringiensis" or a DNA fragment having substantial sequence homology to Bt2.

DETD(68) Straight promotor-gene "fusions" in which only part of the Bt2 coding sequence is used ("truncated Bt2"). Fragments of the Bt2 sequence still encoding an active "toxin" are inserted behind the plant specific promotors: The toxic polypeptides produced in the plant cells using these constructs should have. . .

DETD(71) Straight promotor-gene "fusions" in which a Bt: NPTII "fusion" gene (also referred to at times at Bt2: NPTII) is inserted behind the promotor. "Fusion" genes were constructed, consisting of a fragment of the Bt2 coding sequence (still encoding an active "toxin") "fused" to the coding sequence of the NPTII enzyme. The Bt: NPTII "fusion" genes used here, specify stable "fusion" proteins comprising amino terminal parts of the Bt2 protein "fused" to an intact Neomycin phosphotransferase (NTPII) enzyme. These "fusion" proteins have a specific toxicity comparable to the intact Bt2 protein and retain neomycin phosphotransferase enzyme activity. Thus, expression of the Bt: NPTII "fusion" proteins in plant cells allows direct selection for the production of this protein by isolating Kanamycin resistant (Km.sup.R) transformed cells.. . . to a high level of Kanamycin should identify, among all possible transformations, those which produce high levels of the toxic "fusion" protein. Further, expression of the "fusion" protein by a Bt: NPTII "fusion" gene might have other desirable properties such as stability in plant cells; for example, mRNA may be more stable. Differences in results obtained with these Type IV "fusion" genes might be due to intrinsic differences in the properties of the "fusion" protein expressed as compared to the intact Bt2 protein.

DETD(88) Kronstad et al., J. Bacteriol., 54, p. 419-428 (1983) reported that B.t. berliner 1715 contains two related "toxin" genes which are both located on plasmids. Intact "endotoxin" genes were isolated from a gene bank from total B.t. berliner 1715 plasmid DNA using partial Sau3A digests of plasmid. . . DNA. The pEcoR251 plasmid is a derivative of plasmid pBR322 in which the EcoRI-PuvII fragment has been replaced by a "chimeric" EcoRI endonuclease gene which is "fused" to a P.sub.R promotor fragment derived from plasmid pLK5 (Zabeau and Stanley, EMBO Journal, 1, 1217-1224 (1982)) as depicted in. . .

DETD(135) The previous data suggests that the smallest gene fragment of Bt2, encoding an active "toxin" is contained within the KpnI deletion fragment but extends further than the HindIII site. To map the exact endpoint of the minimal fragment coding for the active "toxin", deletion mutants were constructed which contained N-terminal fragments of decreasing size. To achieve this, we used a strategy which allowed us to construct simultaneously deletion-mutants and translational "fusions" to the NPTII-gene (see Section 7.2.2). The construction of the intermediate plasmid pLBKm25 is outlined in FIG. 18. As shown. . .

DETD(136) As . . . Bal31, cut with SalI, treated with Klenow polymerase and religated (FIG. 19). In this way, the deleted coding region is "fused" to a stopcodon with a minimum of nonsense coding sequence. An overview of the deletion clones is given in FIG.. . . blotting and ELISA for the quantitative detection of Bt2-like polypeptides and in an insect toxicity assay to screen for active "toxin". The results are presented in FIG. 21 and indicate that detection of a stable polypeptide decreases gradually when the endpoint. . .

DETD(141) Since NPTII is a most suitable selection marker in plant engineering, such gene "fusions" could have very promising applications. Indeed when using such NPTII "fusion" proteins to transform plants, a selection for high kanamycin resistance would allow direct selection for a high expression of the "fusion" product. Therefore, "toxin" gene "fusions" with NPTII might be used to transform plants and select for transformed plants expressing high levels of "toxin", by selection for kanamycin resistance.

DETD(170) Previous . . . on the identification of minimal active toxic fragments have shown that this Kpn fragment comprises a (approximately 60 Kd) active "toxin" which exhibits the complete toxic activity of the Bt2 molecule. In the following, we wanted to determine whether the Bt: NPT2 "fusion" protein had still the same degree of toxicity.

DETD(176) 145 . . . concentrations. 8 transformants proved more resistant and were able to grow on concentrations higher than 200 ug/ml of kanamycin. The "fusion" point in all 8 clones was determined by restriction enzyme mapping with an accuracy of 20 bp. Surprisingly 7 out of 8 clones had their "fusion" point around the HindIII site at position 1680 of the Bt gene. One clone (pLBKm860) mapped at position approximately 2050. Although the majority of the deletions were "fused" around position 1800, none of these conferred a higher kanamycin resistant phenotype. The 7 clones which have their "fusion" point positioned around the HindIII site are too short to encode an active "toxin". However, one of the clones (pLBKm860) was:

DETD(186) Table . . . is the result of a cointegration of a receptor Ti plasmid with an intermediate vector. Each intermediate vector contains a "chimeric" "toxin" gene comprising a plant promotor sequence derived from the indicated expression vector and a Bt gene cassette.

DETD(215) This example describes the construction of pHD205, an intermediate vector containing a "chimeric" Bt2 "toxin" gene comprising: the nopaline synthase promotor, the Bt2 "toxin" gene cassette from pHD160 and a DNA fragment containing the 3' untranslated region of the nopaline synthase gene including the polyadenylation site. In the "chimeric" gene the Bt2 gene cassette is oriented such that the expression of the Bt2 protein can be obtained from the. . . are fragments of approximately 6200 bp, 3000 bp, 1800 bp and 920 bp. A recombinant plasmid with the alpha-orientation (the "toxin" gene under the control of the nopaline synthase promotor) is used in subsequent experiments and called pHD205.

DETD(217) This example describes the construction of pHD208. The intermediate vector pHD208 contains a "chimeric" Bt2 "toxin" gene comprising: the promotor from a pea gene encoding a small subunit of ribulose biphosphate carboxylase (Pssu), the Bt2 "toxin" gene cassette from pHD160 and the 3' untranslated region of the octopine synthase gene including the polyadenylation site. The fragments of the "chimeric" gene were assembled in the cloning vector pGV831 as described in this example and as diagrammed in FIG. 29. The. . .

DETD(261) 10. Isolation of plant cells and plants containing the "chimeric" "toxin" gene inserted in their genome

DETD(478) A . . . transformation vectors described herein will contain, stably inserted into their genome, a fragment of newly acquired DNA containing both a "chimeric" Bt "toxin" gene and a marker gene (nos. NPTII). This was confirmed by the results of southern blotting experiments. The new phenotypic traits acquired through this transformation method (expression of Bt "Toxin", antibiotic resistance, nopaline production) will be inherited according to classic Mendelian genetics. To verify stable inheritance of the new traits, F.sub.1 descendants from transformed plants were analysed for the expression of Bt "toxin" and synthesis of nopaline.

DETD(492) TABLE 4

Toxicity of Bt:NPT2 "Fusion" Protein on 3rd Instar
P. brassicae (% Mortality After 4 Days)

"Toxin" dose (ug/ml) Bt protein	0.1	0.2	0.3	0.6	1
Bt2	70	NT.sup.(x)			
		90	NT	100	
Bt:NPT2	NT. . .				

DETD(493) TABLE 5

Toxicity of Intact Bt2 Protein, 60 Kd "Processed"Bt2 Protein (Trypsin Digested) and Bt:NPT2"Fusion" Protein on Larvae of Manduca sexta

% Mortality after 4 days "Toxin" dose: (ng/cm.sup.2)	0	0.67	2	6	18	54	162
130 Kd Bt2	0	0	0	0	3	8	100. . . 20.7 9.4 5.4 2.4
60 Kd Bt2 --		16.3	8.3	6.4	3.9		
Bt:NPT2 --		26.5	15.8	7.7	4.5		

"Toxin" dilutions were applied on artificial diet as described in Section 12. Thirty (30) 1st instar larvae were used per. .

US PAT NO: 5,306,628 [IMAGE AVAILABLE] L10: 25 of 31

BSUM(2) This . . . the host range of insecticidal proteins and/or increasing their toxicity in a certain species. These goals can be achieved by "fusing" an insecticidal protein with another protein segment capable of interacting with the midgut or hindgut epithelium of immature or adult target insects. The present invention relates to the designing of such new "chimeric" proteins having extended host range and/or increased toxicity. More particularly, the invention concerns "chimeric" proteins comprising a first protein segment having insecticidal activity and a second protein segment capable of binding strongly to the. . . insects) to which the first protein segment is not efficiently bound. The first protein segment preferably is a crystal protein (.delta.-"endotoxin") of Bacillus "thuringiensis" (B. "thuringiensis"), or a fragment thereof having insecticidal activity, whereas the second protein segment may, for example, be a surface glycoprotein of an insect nuclear polyhedrosis virus. By combining a B. "thuringiensis" insecticidal crystal protein with another protein segment capable of binding to the midgut or hindgut epithelium of a target insect, the otherwise rather limited host range of B. "thuringiensis" crystal insecticidal proteins can be substantially increased, and the toxicity can be improved. The invention relates to all means and method associated with the production and use of such "chimeric" proteins. The invention also includes other methods for increasing the host range and/or improving the toxicity of insecticidal proteins which do not require the construction of such "chimeric" proteins.

BSUM(9) B. "thuringiensis" is known to produce crystalline inclusions during sporulation. When ingested by the larvae of target insects, these crystalline inclusions are. . . of these crystal proteins are protoxins that are proteolytically converted into smaller toxic polypeptides in the insect midgut. The "activated" "toxin" interacts with the midgut epithelium cells of susceptible insects. According to a recent model, the toxins induce the formation of. . .

BSUM(16) We have surprisingly found that the low efficacy of interaction between certain insecticidal toxins, for example B. "thuringiensis" crystal proteins (Cry proteins, .delta.-endotoxins), and the gut epithelial cells of certain insects can be efficiently improved by providing an additional protein domain of viral origin to the "toxin", which can interact more efficiently with the gut (usually midgut or hindgut) epithelium of the target insect.

BSUM(17) It. . . having high affinity to the lipid components of membranes. This approach that can, for example, be realized by constructing a "chimeric" protein that not only will improve the toxicity by concentrating more of the "toxin" on the midgut epithelial cell surface, but also will confer specificity through its receptor binding domain. Accordingly, via construction of "chimeric" genes of insecticidally active toxins and specific midgut/hindgut binding proteins, "chimeric" "toxin" proteins with increased host range and toxicity can be produced.

DETD(18) Additionally, "chimeric" "toxin" proteins with new insecticidal properties and/or increased toxicity generated by this approach can be expressed in commercially important plants thus. . . making them resistant to variety of insect pests instead of few. Utilizing the Ti plasmids which carry CaMV35S promoter, these "chimeric" proteins can be expressed in plants like tomato, tobacco, cotton, potato etc., [Vaeck, M. et al., Nature, 327, 6125, 33-37. . .

DETD(25) According to a preferred embodiment of the invention, DNA sequences encoding B. "thuringiensis" .delta.-endotoxins and the gp64 viral membrane glycoprotein of ACNPV are operably linked, and the combined DNA sequence is expressed in host organisms to produce "chimeric" Bt/gp64 "chimeric" "toxin" proteins.

DETD(26) ACNPV . . . of currently available microbial insecticides. The AcNPV gp64 receptor binding domain interacts with its specific host midgut receptors, whereby more "chimeric" "toxin" is concentrated on the midgut epithelial cell surface, and toxicity is improved. Even more importantly, by providing an additional receptor. . . sufficiently toxic. In other words, gp64 gene sequences can be used as midgut targeting signals for bacterial endotoxins, including Bt "endotoxin".

DETD(27) According to another preferred embodiment of the invention, a B. "thuringiensis" "toxin" is combined with a 27.3-kDA protein of B. "thuringiensis" (from subspecies israelensis - Bti, and morrisoni - Btm) which is known to have high affinity for the lipid portion. . .

DETD(46) Although the invention is illustrated by construction of "chimeric" proteins, utilizing the approach provided by the present invention, other methods can also be designed for increasing the host-range of. . . midgut binding protein and an insecticidal protein on its surface could be used as a delivery vehicle for insect "toxin" proteins. Alternatively, even a baculovirus infected insect cell which has both midgut binding protein and an insecticidal protein on its. . . surface, could be used as delivery vehicle for insect toxins. This can, for example, be designed by expressing the insecticidal "toxin" protein as an integral transmembrane protein using a baculovirus vector. This process generates an infected insect cell containing both the. . . dried or lyophilized are fed to caterpillars, gp64 would bind strongly to midgut epithelial calls thus bringing the neighboring insecti "toxin" protein to reach or interact with its target. Thus surface proteins of viruses could be used as deliver vehicles or. . .

DETD(61) One of the tools required for this gene "fusion" study is to obtain the genes coding for delta endotoxins from strains which are toxic to lepidopterans and coleopteran beetles. We have chosen Coleopteran BT "toxin" Bacillus "thuringiensis" tenebrionis, Btt) over Lepidopteran BT "toxin" for several reasons. One among them is, since the gp64 is from a virus which infects exclusively lepidopteran hosts, when "fused" with the coleopteran "toxin", it will be easier to assay the "chimeric" "toxin" protein for its newly acquired toxicity against lopidopteran larvae (Trichoplusia ni). For obtaining the gone coding for the coleopteran "toxin", Bacillus thuringionsis tenebrionis (Btt) was obtained from Safer Inc., Newton, Mass. utilizing the published sequence of Btt protein [Hofte, H. . . . following sequences: 26 mer - 5'-AAGCTTACAGAGAAATACACGAGGGC-3'; 31 mer-5'AAGCTTAATTAAAGATAATATCTTTGAATT G-3'; were designed and made in order to synthesize the 2.98 kbp coleopteran "toxin" gene using the polymerase chain reaction (PCR) technique. Although the PCR experiments were initially successful, later experiments failed due to. . . screen the colonies of Btt-pUC13 recombinant library. Total DNA (both chromosomal and plasmid) was isolated from the bacterial strain Bacillus "thuringiensis" tenebrionis (Btt). (This strain was obtained from Safer Inc. Isolated bacterial DNA was then digested with the restriction enzyme HindIII. . . the liberated DNA onto the paper. The nitrocellulass paper was then subjected to DNA hybridization using the radiolabeled (32P) Btt "toxin" specific oligonucleactides (26 mer & 31 mer) as the probes. Three colonies that hybridized to the probe (pUC7-1, pUcg-10 and. . .

DETD(65) Although pUC 12-7 was toxic to coleopteran larvae (Table 1) , we could not detect the 66kD Or 72KD Btt "toxin" protein on a comassio-Blue stained SDS-PAGE. This indicated to us that the Btt "toxin" is made in very small amounts since the expression is driven by Btt promoter. Since future experiments also (Btt/gp64 "fusions") require high level expression, we decided to express the Btt protein in large amounts in E. coli using an

expression plasmid pT7-7 which has a strong T7 bacteriophage promoter (FIGS. 7 and 13). Initially, the Btt *toxin* gene from pUC12-7 was first subcloned into pT7-7 vector (a T7 promoter based vector for expressing foreign genes) and the coleopteran *toxin* was expressed in vivo in E. coli both as a *fusion* protein with T7 phage gene 10 protein amino terminus (pT7-SX12 and pT7-X9) and as a non-*fusion* native Btt *toxin* protein (pT7-4-4). All these recombinant plasmids were transformed into a E. coli strain BL21 [Studier, F. W., & B. A. . . . of lacZ promoter and the recombinant Btt can be expressed by inducing with IPTG. These recombinant plasmids expressed the Btt *toxin* to higher levels than pUC12-7 and were also toxic to coleopteran larvae (FIG. 8 & Table 2).

DETD(68) After constructing the above recombinant plasmids which expresses the Btt *toxin* proteins in E. coli, Btt/gp64 gene *fusions* were constructed. The strategy for these recombinant DNA *fusions* are shown in FIG. 9. A unique Xmn1 restriction site at the coding region of the carboxyl terminus of Btt. . . restriction sites was positioned near the Xmnl site through DNA legation. Utilizing the polylinker restriction sites, three different Btt/gp64 gene *fusions* (pFAv10, pFX7 & pFAc13) were constructed and are shown in FIG. 9. All these recombinant plasmids were transformed into a. . . coding for T7 RNA polymerase protein. This gene is under the control of lacZ promoter and the recombinant Btt/gp64 gene *fusions* can be expressed by inducing with IPTG. Immunoblotting experiments with these Rtt/gp64 *fusion* proteins indicate that all the *fusion* proteins were expressed but at a lower level when compared to Btt itself (FIG. 10).

DETD(70) a) Toxicity bioassays with Btt/gp64 *fusion* proteins were carried out against Trichoplusia ni neonate larvae. Basically these experiments were done with lima beans artificial diet. We grew large batches of E. coli cultures which expresses these *fusion* proteins and identical amounts of control E. coli pT7-7 and E. coli Btt/gp64 pFAv10 were mixed respectively with the identical. . . the mortality due to toxicity was recorded. Control pT7-7 showed 34% mortality while the pFAv10 which expresses the 125kD Btt/gp64 *fusion* protein exhibited 551 mortality. The experiments were done only with one high dose. When observed, the surviving larvae on the Btt/gP64 *fusion* diet were comparatively small, sick and lethargic. The pictures of these surviving larvae and is shown in FIG. 11. Also,. . . the midgut was observed in the Btt/gp64 fed larvae when compared to the control pT7-7 fed larvae, indicating that the *chimeric* Btt/gp64 *toxin* has interacted and disturbed the ionic flow across the membrane. Histopathological studies are in progress to determine the exact nature of the gut damage. In addition, earlier toxicity bioassay experiments with slightly lower concentrations of Btt/gp64 *fusion* proteins also exhibited this toxicity. These experiments clearly indicate that the Btt/gp64 *fusion* protein has acquired the new toxicity is towards lepidopteran larvae and might have caused the gut damage.

DETD(74) These results indicate that the Btt-gp64 *fusion* protein are toxic to lepidopteran aliothis larvae and among them PFAv10 is most toxic (see FIG. 23). It should be noted that in all the *fusion* protein inclusion body preparations (pFAv10, pFx7 and pFAc13) only 15% of the total proteins are undergraded full length *fusion* protein molecules because of protein degradation. However, in the control pSX12T non-*fusion* Btt protein, approximately 80% of the total protein is undegraded full length Btt protein molecules (see FIG. 24). Thus the Btt-gp64 *fusion* proteins possess higher toxicity against lepidopteran larvae than the non-*fusion* coleopteran Btt *toxin* protein. Additional precise protein engineering had to be done in order to further increase the toxicity of Btt-gp64 *fusion* proteins against the lepidopteran larvae. Thus these experiments prove the concept that an insect midgut binding domain of a protein. . .

We claim;
1. A *chimeric* protein having insecticidal properties, comprising: a first protein domain comprising a B. *thuringiensis* crystal protein having insecticidal properties; and a second protein domain *fused* to said first protein domain, said second domain comprising an insect gut binding polypeptide of viral origin.

CLMS(3) 3. The protein of claim 1, wherein said first domain is a B. *thuringiensis* subsp. israelensis domain.

CLMS(4) 4. The protein of claim 3 wherein said first domain is the approximately 72 kd crystal protein of B. *thuringiensis* subsp. israelensis.

CLMS(5) 5. The protein of claim 1, wherein said first domain is a B. *thuringiensis* subsp. tenebriosis domain.

US PAT NO: 5,254,799 [IMAGE AVAILABLE] L10: 26 of 31
TITLE: Transformation vectors allowing expression of Bacillus *thuringiensis* endotoxins in plants

ABSTRACT: Novel transformation vectors containing novel *chimeric* genes allow the introduction of exogenous DNA fragments coding for polypeptide toxins produced by Bacillus *thuringiensis* or having substantial sequence homology to a gene coding for a polypeptide *toxin* as described herein and expression of the *chimeric* gene in plant cells and their progeny after integration into the plant cell genome. Transformed plant cells and their progeny exhibit stably inherited polypeptide *toxin* expression useful for protecting said plant cells and their progeny against certain insect pests and in controlling said insect pests.

BSUM(3) This . . . the use of genetic engineering techniques in the modification of plants. More particularly, it concerns introduction and integration of a *chimeric* gene coding for a polypeptide *toxin* produced by Bacillus *thuringiensis* or having substantial sequence homology to a *toxin* gene described below in plant cells and obtaining an insect controlling level of expression of said polypeptide *toxin* intracellularly by transformed plant cells and their progeny.

BSUM(8) Bacillus *thuringiensis* (referred to at times herein as B.t.) bacteria includes approximately 19 known varieties that produce polypeptide toxins which form parasporal. . . by insect larvae, the crystals are solubilized and processed in the insect midgut to yield at least one active polypeptide *toxin* which is believed to act on the midgut cell membrane. Studies have revealed that individual crystal polypeptides exhibit insecticidal activity.. . .

BSUM(13) It is one object of this invention to provide novel *chimeric* genes coding for the polypeptide *toxin* produced by Bacillus *thuringiensis*, or coding for a polypeptide *toxin* having substantial sequence homology to a *toxin* gene described herein. The *chimeric* genes' plant regulatory sequences direct expression in transformed plant cells.

BSUM(20) (b) at least one DNA fragment coding for a polypeptide *toxin* produced by Bacillus *thuringiensis* or having substantial sequence homology thereto.

BSUM(27) (ii) at least one DNA fragment coding for a polypeptide *toxin* produced by Bacillus *thuringiensis* or at least one DNA fragment having substantial sequence homology thereto.

BSUM(31) (b) at least one DNA fragment coding for a polypeptide *toxin* produced by Bacillus *thuringiensis*, or at least one DNA fragment having substantial sequence homology thereto.

BSUM(35) Transformed plant cells and their progeny intracellularly express a polypeptide *toxin* substantially similar to the polypeptide toxins produced by Bacillus *thuringiensis* and are substantially toxic to certain insects. Transformed plant cells and their progeny may be used in controlling said insects.

DETD(7) (1) isolation of at least one DNA fragment from Bacillus *thuringiensis* coding for a polypeptide *toxin* by digestion of bacterial DNA and inserting the mixture of DNA fragments obtained into a cloning vehicle harbored in a. . .

DETD(25) Transformed plant cells and their progeny should express a polypeptide *toxin* substantially similar to polypeptide toxins being produced by Bacillus *thuringiensis* or a DNA fragment having substantial sequence homology to Bt2.

DETD(67) Straight promotor-gene *fusions* in which only part of the Bt2 coding sequence is used (*truncated Bt2*). Fragments of the Bt2 sequence still encoding an active *toxin* are inserted behind the plant specific promotors: The toxic polypeptides produced in the plant cells using these constructs should have. . .

DETD(70) Straight promotor-gene *fusions* in which a Bt:NPTII *fusion* gene (also referred to at times at Bt2:NPTII) is inserted behind the promotor. *Fusion* genes were constructed, consisting of a fragment of the Bt2 coding sequence (still encoding an active *toxin*) *fused* to the coding sequence of the NPTII enzyme. The Bt:NPTII *fusion* genes used here, specify stable *fusion* proteins comprising amino terminal parts of the Bt2 protein *fused* to an intact Neomycin phosphotransferase (NTPII) enzyme. These *fusion* proteins have a specific toxicity comparable to the intact Bt2 protein and retain neomycin phosphotransferase enzyme activity. Thus, expression of the Bt:NPTII *fusion* proteins in plant cells allows direct selection for the production of this protein by isolating Kanamycin resistant (Km.RTM.) transformed cells.. . . to a high level of Kanamycin should identify, among all possible transformations, those which produce high levels of the toxic *fusion* protein. Further, expression of the *fusion* protein by a Bt:NPTII *fusion* gene might have other desirable properties such as stability in plant cells; for example, mRNA may be more stable. Differences in results obtained with these Type IV *fusion* genes might be due to intrinsic differences in the properties of the *fusion* protein expressed as compared to the intact Bt2 protein.

DETD(87) Kronstad et al., J. Bacteriol., 54, p. 419-428 (1983) reported that B.t. berliner 1715 contains two related *toxin* genes which are both located on plasmids. Intact *endotoxin* genes were isolated from a gene bank from total B.t. berliner 1715 plasmid DNA using partial Sau3A digests of plasmid. . . DNA. The pEcoR251 plasmid is a derivative of plasmid pBR322 in which the EcoRI-PuvII fragment has been replaced by a *chimeric* EcoRI endonuclease gene which is *fused* to a P.sub.R promotor fragment derived from plasmid pLK5 (Zabeau and Stanley, EMBO Journal, 1, 1217-1224 (1982)) as depicted in. . .

DETD(134) The previous data suggests that the smallest gene fragment of Bt2, encoding an active *toxin* is contained within the KpnI deletion fragment but extends further than the HindIII site. To map the exact endpoint of the minimal fragment coding for the active *toxin*, deletion mutants were constructed which contained N-terminal fragments of decreasing size. To achieve this, we used a strategy which allowed us to construct simultaneously deletion-mutants and translational *fusions* to the NPTII-gene (see Section 7.2.2). The construction of the intermediate plasmid pLBKm25 is outlined in FIG. 18. As shown. . .

DETD(135) As . . . Ba131, cut with SalI, treated with Klenow polymerase and religated (FIG. 19). In this way, the deleted coding region is *fused* to a stopcodon with a minimum of nonsense coding sequence. An overview of the deletion clones is given in FIG.. . . blotting and ELISA for the quantitative detection of Bt2-like polypeptides and in an insect toxicity assay to screen for active *toxin*. The results are presented in FIG. 21 and indicate that detection of a stable polypeptide decreases gradually when the endpoint. . .

DETD(140) Since NPTII is a most suitable selection marker in plant engineering, such gene *fusions* could have very promising applications. Indeed when using such NPTII *fusion* proteins to transform plants, a selection for high kanamycin resistance would allow direct selection for a high expression of the *fusion* product. Therefore, *toxin* gene *fusions* with NPTII might be used to transform plants and select for transformed plants expressing high levels of *toxin*, by selection for kanamycin resistance.

DETD(169) Previous . . . on the identification of minimal active toxic fragments have shown that this Kpn fragment comprises a (approximately 60 Kd) active *toxin* which exhibits the complete toxic activity of the Bt2 molecule. In the following, we wanted to determine whether the Bt:NPT2 *fusion* protein had still the same degree of toxicity.

DETD(175) 145 . . . concentrations. 8 transformants proved more resistant and were able to grow on concentrations higher than 200 ug/ml of kanamycin. The *fusion* point in all 8 clones was determined by restriction enzyme mapping with an accuracy of 20 bp. Surprisingly 7 out of 8 clones had their *fusion* point around the HindIII site at position 1680 of the Bt gene. One clone (pLBKm860) mapped at position approximately 2050. Although the majority of the deletions were *fused* around position 1800, none of these conferred a higher kanamycin resistant phenotype. The 7 clones which have their *fusion* point positioned around the HindIII site are too short to encode an active *toxin*. However, one of the clones (pLBKm860) was:

DETD(185) Table . . . is the result of a cointegration of a receptor Ti plasmid with an intermediate vector. Each intermediate vector contains a *chimeric* *toxin* gene comprising a plant promotor sequence derived from the indicated expression vector and a Bt gene cassette.

DETD(214) This example describes the construction of pHD205, an intermediate vector containing a *chimeric* Bt2 *toxin* gene comprising: the nopaline synthase promotor, the Bt2 *toxin* gene cassette from pHD160 and a DNA fragment containing the 3' untranslated region of the nopaline synthase gene including the polyadenylation site. In the *chimeric* gene the Bt2 gene cassette is oriented such that the expression of the Bt2 protein can be obtained from the. . . are fragments of approximately 6200 bp, 3000 bp, 1800 bp and 920 bp. A recombinant plasmid with the alpha-orientation (the *toxin* gene under the control of the nopaline synthase promotor) is used in subsequent experiments and called pHD205.

DETD(216) This example describes the construction of pHD208. The intermediate vector pHD208 contains a *chimeric* Bt2 *toxin* gene comprising: the promotor from a pea gene encoding a small subunit of ribulose biphosphate carboxylase (Pssu), the Bt2 *toxin* gene cassette from pHD160 and the 3' untranslated region of the octopine synthase gene including the polyadenylation site. The fragments of the *chimeric* gene were assembled in the cloning vector pGV831 as described in this example and as diagrammed in FIG. 29. The. . .

DETD(260) 10. Isolation of plant cells and plants containing the *chimeric* *toxin* gene inserted in their genome

DETD(475) A . . . transformation vectors described herein will contain, stably inserted into their genome, a fragment of newly acquired DNA containing both a *chimeric* Bt *toxin* gene and a marker gene (nos, NPTII). This was confirmed by the results of southern blotting experiments. The new phenotypic traits acquired through this transformation method (expression of Bt *Toxin*, antibiotic resistance, nopaline production) will be inherited according to classic Mendelian genetics. To verify stable inheritance of the new traits, F.sub.1 descendants from transformed plants were analysed for the expression of Bt *toxin* and synthesis of nopaline.

DETD(489) TABLE 4

Toxicity of Bt:NPT2 *Fusion* Protein on 3rd Instar
P. brassicae (% Mortality After 4 Days)

	Toxin dose (ug/ml)				
Bt protein	0.1	0.2	0.3	0.6	1
Bt2	70				
NT.sup.(x)		90	NT	100	
Bt:NPT2	NT	80. . .			

DETD(490) TABLE 5

Toxicity of Intact Bt2 Protein, 60 Kd *Processed* Bt2 Protein (Trypsin Digested) and Bt:NPT2 *Fusion* Protein on Larvae of Manduca sexta

% Mortality after 4 days								
Toxin dose: (ng/cm.sup.2)	0	0.67	2	6	18	54	162	
130 Kd Bt2	0	0	0	0	3	8. . .	9.4	5.4 2.4
60 Kd Bt2								
--		16.3	8.3		6.4 3.9			
Bt:NPT2 --			26.5	15.8		7.7 4.5		

Toxin dilutions were applied on artificial diet as described in Section 12. Thirty (30) 1st instar larvae were used per. . .
We claim:
1. A plant cell susceptible to transformation by Agrobacterium, the genome of which contains a *chimeric* gene comprising: a) a first DNA fragment that encodes a N-terminal fragment of approximately 60-80 kD, derived from DNA encoding a Bacillus *thuringiensis* insecticidal crystal protein of approximately 130 kD which has been truncated; and b) a promoter region and a 3' non-translated region. . . region; the promoter and 3' non-translated regions allowing the first DNA fragment to be expressed in the cell; whereby the *chimeric* gene can be expressed in the cell as an insect controlling amount of an insecticidal Bacillus *thuringiensis* polyopeptide *toxin* with toxicity to Lepidoptera insects.

CLMS(6) 6. A plant cell susceptible to transformation by Agrobacterium, the genome of which contains a *chimeric* gene comprising: a) a first DNA fragment that encodes a N-terminal fragment of approximately Kd, derived from DNA encoding. . . region; the promoter and 3' non-translated regions allowing the first DNA fragment to be expressed in the cell; whereby the *chimeric* gene can be expressed in the cell as an insect controlling amount of an insecticidal Bacillus *thuringiensis* polypeptide *toxin* with toxicity to Lepidoptera insects.

CLMS(7) 7. . . . of claim 6 wherein the first DNA fragment encodes: a truncated portion of a 130 kD crystal protein of Bacillus *thuringiensis* berliner 1715, a truncated portion of a 130 kD crystal protein of Bacillus *thuringiensis* kurstaki; or a truncated portion of a 130 kD crystal protein of Bacillus *thuringiensis* sotto.

CLMS(11) 11. The cell of claim 6 wherein the *chimeric* gene also comprises a second DNA fragment which encodes an enzyme capable of being expressed in the cell and the expression of which can be identified in the cell; the second DNA fragment being *fused* to the first DNA fragment so that the first and second DNA fragments encode a *fusion* polypeptide; whereby an identification of expression of the second DNA fragment in the cell provides an identification of expression of. . .

CLMS(14) 14. The cell of claim 11 wherein the second DNA fragment is *fused* to the first DNA fragment containing a trypsin cleavage site.

US PAT NO: 5,177,308 [IMAGE AVAILABLE] L10: 27 of 31
ABSTRACT: Transgenic plants have been created which express an insect-specific *toxin* from a scorpion. The *chimeric* inheritable trait produced conditions of toxicity in the plant cells of toxicity to certain insects upon ingestion of plant tissues. The inheritable trait has also been cross-bred to plants transgenic to the Bacillus *thuringiensis* delta-*endotoxin* to produce plants having two independent insect-specific *toxin* traits. Insect

feeding trails revealed additive toxic effects. A generalized approach for developing other insecticidal toxins as candidates for insertion. . .

BSUM(5) Biological . . . several of these criteria For example, there have been several products based on the use of various forms of the delta-"endotoxin" produced by the soil dwelling microorganism Bacillus "thuringiensis" (B.t.) as insecticidal agents. This polypeptide "toxin" has been found to be specifically toxic to Lepidopteran insects, and has been used for many years commercially as a foliar applied insecticide. It has also recently been found that various forms of the B.t. "toxin" can be toxic to insects, when expressed inside the tissues of plants on which the insects feed. This is the. . .

DETD(3) One particular "toxin" which has been discovered here and is described in further detail below is a polypeptide "toxin" which was discovered as a constituent of the venom produced by a North African scorpion Androctonus australis. This "toxin" has been one which was found to be toxic to insects in both in vitro and in vivo tests. Other. . . by similar screening and testing. In particular, the phenomenon of insect toxicity uncovered and found to be effective with the "toxin" disclosed herein was developed by a rational plan to respond to a perceived need to find other traits which could. . . protection against insect predation. It has previously been demonstrated, notably with the toxins produced by the soil dwelling microorganism Bacillus "thuringiensis", that insecticidal toxins can successfully be produced in plant cells so as to render those plants toxic to insects which. . . toxins, which aided in the selection of a particular protein the toxicity of which could be screened. Accordingly, the scorpion "toxin" represented a class of agents which could be investigated for possible insecticidal "toxin" activity. Other target predators of insects include any animals which rely on a peptide "toxin" to either incapacitate or kill their insect prey.

DETD(4) It must be remembered that to be a candidate for genetic insertion into plants, a "toxin" should ideally meet several constraints. One constraint, at least at present given the level of skill in the art of genetically engineering plants, is that the "toxin" should preferably be a peptide which can be synthesized by a single gene trait which can be inserted into plant. . . be possible to insert genes coding for enzymes which catalyze the synthesis of non-peptide toxins. Another constraint is that the "toxin" should be selected so as to be relatively specific in its activity. Many toxins are active broadly against most animals.. . . candidates for genetic engineering into plants to be used for human or animal food. However, the developing capacity to construct "chimeric" genes to express peptides in plants in a tissue-specific manner raises the possibility of using broader spectrum toxins, since it. . . will produce the toxins only in plant tissues that will not be used as animal or human food. Nevertheless, optimal "toxin" candidate would be a "toxin" which is uniquely toxic to insects, but which is minimally or not at all toxic to any other classes of. . . the plants. In particular, it is desired that there be no toxicity to mammals, so that the insertion of the "toxin" into the cells of plants still results in plants which have unchanged nutritive value to humans or to domestic animals.

DETD(7) Once a "toxin", such as AalT, has been selected, it is then necessary to prepare a "chimeric" expression cassette suitable for expressing the peptide in the cells of target plants. There are a number of ways in which such a "chimeric" expression cassette can be constructed, as is known to those of ordinary skill in the art. At a minimum, the. . .

DETD(29) FIG. . . . plant T3219, an R2 plant which had within it a homozygous insertion of the single BTS gene expressing the Bacillus "thuringiensis" delta-"endotoxin" in insect toxic doses, and in addition plant T3219 was self-pollinated. Prior to feeding trials all of the progeny of. . .

DETD(41) As in Examples 1 and 2 demonstrating synthesis of genes encoding the AalT and BeIT1 peptides, respectively, a "chimeric" "toxin" gene was constructed to enable expression of BeIT2 in plants. Based on the most frequently used codons in plants (FIG.. . .

CLMS(2) 2. . . . in plant cells, one genetic construction including a coding region coding for the expression in plant cells of insect specific "toxin" AalT, the other genetic construction including a coding region coding for the expression in plant cells of the Lepidopteran specific delta "endotoxin" gene from Bacillus "thuringiensis", the linked genetic constructions effective to express in the cells of the plant sufficient amounts of AalT to be lethal upon ingestion by Heliothis zea and sufficient amounts of the delta "endotoxin" to be toxic upon ingestion by Manduca sexta.

US PAT NO: 5,143,905 [IMAGE AVAILABLE] L10: 28 of 31

BSUM(2) This . . . the host range of insecticidal proteins and/or increasing their toxicity in a certain species. These goals can be achieved by "fusing" an insecticidal protein with another protein segment capable of interacting with the midgut or hindgut epithelium of immature or adult target insects. The present invention relates to the designing of such new "chimeric" proteins having extended host range and/or increased toxicity. More particularly, the invention concerns "chimeric" proteins comprising a first protein segment having insecticidal activity and a second protein segment capable of binding strongly to the. . . insects) to which the first protein segment is not efficiently bound. The first protein segment preferably is a crystal protein (.delta.-"endotoxin") of Bacillus thuringiensis (B. "thuringiensis"), or a fragment thereof having insecticidal activity, whereas the second protein segment may, for example, be a surface glycoprotein of an insect nuclear polyhedrosis virus. By combining a B. "thuringiensis" insecticidal crystal protein with another protein segment capable of binding to the midgut or hindgut epithelium of a target insect, the otherwise rather limited host range of B. "thuringiensis" crystal insecticidal proteins can be substantially increased, and the toxicity can be improved. The invention relates to all means and method associated with the production and use of such "chimeric" proteins. The invention also includes other methods for increasing the host range and/or improving the toxicity of insecticidal proteins which do not require the construction of such "chimeric" proteins.

BSUM(9) B. "thuringiensis" is known to produce crystalline inclusions during sporulation. When ingested by the larvae of target insects, these crystalline inclusions are. . . of these crystal proteins are protoxins that are proteolytically converted into smaller toxic polypeptides in the insect midgut. The "activated" "toxin" interacts with the midgut epithelium cells of susceptible insects. According to a recent model, the toxins induce the formation of. . .

BSUM(16) We have surprisingly found that the low efficacy of interaction between certain insecticidal toxins, for example B. "thuringiensis" crystal proteins (Cry proteins, .delta.-endotoxins), and the gut epithelial cells of certain insects can be efficiently improved by providing an additional protein domain of viral origin to the "toxin", which can interact more efficiently with the gut (usually midgut or hindgut) epithelium of the target insect.

BSUM(18) This approach that can, for example, be realized by constructing a "chimeric" protein that not only will improve the toxicity by concentrating more of the "toxin" on the midgut epithelial cell surface, but also will confer specificity through its receptor binding domain. Accordingly, via construction of "chimeric" genes of insecticidally active toxins and specific midgut/hindgut binding proteins, "chimeric" "toxin" proteins with increased host range and toxicity can be produced.

DETD(19) Additionally, "chimeric" "toxin" proteins with new insecticidal properties and/or increased toxicity generated by this approach can be expressed in commercially important plants thus. . . making them resistant to variety of insect pests instead of few. Utilizing the Ti plasmids which carry CaMV35S promoter, these "chimeric" proteins can be expressed in plants like tomato, tobacco, cotton, potato etc., [Vaeck, M. et al., Nature, 327, 6125, 33-37. . .

DETD(26) According to a preferred embodiment of the invention, DNA sequences encoding B. "thuringiensis" .delta.-endotoxins and the gp64 viral membrane glycoprotein of AcNPV are operably linked, and the combined DNA sequence is expressed in host organisms to produce "chimeric" Bt/gp64 "chimeric" "toxin" proteins.

DETD(27) AcNPV . . . of currently available microbial insecticides. The AcNPV gp64 receptor binding domain interacts with its specific host midgut receptors, whereby more "chimeric" "toxin" is concentrated on the midgut epithelial cell surface, and toxicity is improved. Even more importantly, by providing an additional receptor binding domain to the Bt .delta.-"endotoxin", specificity is improved, and the host range of Bt toxins can be extended to insects to which they are not. . . sufficiently toxic. In other words, gp64 gene sequences can be used as midgut targeting signals for bacterial endotoxins, including Bt "endotoxin".

DETD(28) According to another preferred embodiment of the invention, a B. "thuringiensis" "toxin" is combined with a 27.3-kDA protein of B. "thuringiensis" (from subspecies israelensis - Bti, and morrisoni - Btm) which is known to have high affinity for the lipid portion. . .

DETD(47) Although the invention is illustrated by construction of "chimeric" proteins, utilizing the approach provided by the present invention, other methods can also be designed for increasing the host-range of. . . both midgut binding protein and an insecticidal protein on its surface could be used as a delivery vehicle for insect "toxin" proteins. Alternatively, even a baculovirus infected insect cell which has both midgut binding protein and an insecticidal protein on its. . . surface, could be used as delivery vehicle for insect toxins. This can, for example, be designed by expressing the insecticidal "toxin" protein as an integral transmembrane protein using a baculovirus vector. This process generates an infected insect cell containing both the. . . dried or lyophilized) are fed to caterpillars, gp64 would bind strongly to midgut epithelial cells thus bringing the neighboring insecticidal "toxin" protein to reach or interact with its target. Thus surface proteins of viruses could be used as delivery vehicles or. . .

DETD(62) One of the tools required for this gene "fusion" study is to obtain the genes coding for delta endotoxins from strains which are toxic to lepidopterans and coleopteran beetles. We have chosen Coleopteran BT "toxin" Bacillus "thuringiensis" tenebrionis, Btt) over Lepidopteran BT "toxin" for several reasons. One among them is, since the gp64 is from a virus which infects exclusively lepidopteran hosts, when "fused" with the coleopteran "toxin", it will be easier to assay the "chimeric" "toxin" protein for its newly acquired toxicity against lepidopteran larvae (Trichoplusia ni). For obtaining the gene coding for the coleopteran "toxin", Bacillus "thuringiensis" tenebrionis (Btt) was obtained from Safer Inc., Newton, Mass. utilizing the published sequence of Btt protein [Hofte, H. et al.,. . . - 3'; 3 1 m e r -5'AAGCTTAATTAAAGATAATATCTTTGAATTG-3' were designed and made in order to synthesize the 2.98 kbp coleopteran "toxin" gene using the polymerase chain reaction (PCR) technique. Although the PCR experiments were initially successful, later experiments failed due to. . . for PCR were used as probes to screen the colonies chromosomal and plasmid) was isolated from the bacterial strain Bacillus "thuringiensis" tenebrionis (Btt). (This strain was obtained from Safer Inc. Isolated bacterial DNA was then digested with the restriction enzyme HindIII. . . the liberated DNA onto the paper. The nitrocellulose paper was then subjected to DNA hybridization using the radiolabeled (32P) Btt "toxin" specific oligonucleotides (26mer & 31mer) as the probes. Three colonies that hybridized to the probe (pUC7-1, pUC9-10 and pUC12-7) were. . .

DETD(66) Although pUC 12-7 was toxic to coleopteran larvae (Table 1), we could not detect the 66kD 0r 72KD Btt "toxin" protein on a comassie-Blue stained SDS-PAGE. This indicated to us that the Btt "toxin" is made in very small amounts since the expression is driven by Btt promoter. Since future experiments also (Btt/gp64 "fusions") require high level expression, we decided to express the Btt protein in large amounts in E. coli using an expression plasmid pT7-7 which has a strong T7 bacteriophage promoter (FIGS. 7 and 13). Initially, the Btt "toxin" gene from pUC12-7 was first subcloned into pT7-7 vector (a T7 promoter based vector for expressing foreign genes) and the coleopteran "toxin" was expressed in vivo in E. coli both as a "fusion" protein with T7 phage gene 10 protein amino terminus (pT7-SX12 and pT7-X9) and as a non-"fusion" native Btt "toxin" protein (pT7-4-4). All these recombinant plasmids were transformed into a E. coli strain BL21 [Studier, F. W., & B. A. . . of lacZ promoter and the recombinant Btt can be expressed by inducing with IPTG. These recombinant plasmids expressed the Btt "toxin" to higher levels than pUC12-7 and were also toxic to coleopteran larvae (FIG. 8 & Table 2).

DETD(71) After constructing the above recombinant plasmids which expresses the Btt "toxin" proteins in E. coli, Btt/gp64 gene "fusions" were constructed. The strategy for these recombinant DNA "fusions" are shown in FIG. 9. A unique Xmnl restriction site at the coding region of the carboxyl terminus of Btt. . . restriction sites was positioned near the Xmnl site through DNA ligation. Utilizing the polylinker restriction sites, three different Btt/gp64 gene "fusions" (pFAv10, pFX7 & pFAc13) were constructed and are shown in FIG. 9. All these recombinant plasmids were transformed into a. . . coding for T7 RNA polymerase protein. This gene is under the control of lacZ promoter and the recombinant Btt/gp64 gene "fusions" can be expressed by inducing with IPTG. Immunoblotting experiments with these Btt/gp64 "fusion" proteins indicate that all the "fusion" proteins were expressed but at a lower level when compared to Btt itself (FIG. 10).

DETD(73) a) Toxicity bioassays with Btt/gp64 "fusion" proteins were carried out against Trichoplusia ni neonate larvae. Basically these experiments were done with lima beans artificial diet. We grew large batches of E. coli cultures which expresses these "fusion" proteins and identical amounts of control E. coli pT7-7 and E. coli Btt/gp64 pFAv10 were mixed respectively with the identical. . . the mortality due to toxicity was recorded. Control pT7-7 showed 34% mortality while the pFAv10 which expresses the 125kD Btt/gp64 "fusion" protein exhibited 55% mortality. The experiments were done only with one high dose. When observed, the surviving larvae on the Btt/gp64 "fusion" diet were comparatively small, sick and lethargic. The pictures of these surviving larvae and is shown in FIG. 11. Also,. . . the midgut was observed in the Btt/gp64 fed larvae when compared to the control pT7-7 fed larvae, indicating that the "chimeric" Btt/gp64 "toxin" has interacted and disturbed the ionic flow across the membrane. Histopathological studies are in progress to determine the exact nature of the gut damage. In addition, earlier toxicity bioassay experiments with slightly lower concentrations of Btt/gp64 "fusion" proteins also exhibited this toxicity. These experiments clearly indicate that the Btt/gp64 "fusion" protein has acquired the new toxicity towards lepidopteran larvae and might have caused the gut damage.

DETD(77) These results indicate that the Btt-gp64 "fusion" protein are toxic to lepidopteran Heliothis larvae and among them pFAv10 is most toxic (see FIG. 23). It should be noted that in all the "fusion" protein inclusion body preparations (pFAv10, pFx7 and pFAc13) only 15% of the total proteins are undegraded full length "fusion" protein molecules because of protein degradation. However, in the control pSX12T non-"fusion" Btt protein, approximately 80% of the total protein is undegraded full length Btt protein molecules (see FIG. 24). Thus the Btt-gp64 "fusion" proteins possess higher toxicity against lepidopteran larvae than the nonfusion coleopteran Btt "toxin" protein. Additional precise protein engineering had to be done in order to further increase the toxicity of Btt-gp64 "fusion" proteins against the lepidopteran larvae. Thus these experiments prove the concept that an insect midgut binding domain of a protein. . .

CLMS(2) 2. The method of claim 1 wherein said targeting protein or protein domain being originated from a source other than Bacillus "thuringiensis".

CLMS(6) 6. The method of claim 5 wherein said insecticidal protein is a crystal protein of Bacillus "thuringiensis" (B. "thuringiensis"), or a fragment thereof having insecticidal activity.

CLMS(7) 7. The method of claim 6 wherein said insecticidal protein is the crystal protein of a Coleopteran-specific B. "thuringiensis".

CLMS(8) 8. The method of claim 7 wherein said insecticidal protein is the toxic domain of a B. "thuringiensis" subsp. tenebriosis crystal protein.

CLMS(15) 15. The method of claim 14 wherein said insecticidal protein is a crystal protein of Bacillus "thuringiensis" (B. "thuringiensis") or a fragment thereof having insecticidal activity.

CLMS(16) 16. The method of claim 15 wherein said insecticidal protein comprises the toxic domain of a B. "thuringiensis" tenebriosis crystal protein.

CLMS(17) 17. The method of claim 15 wherein said bacterial protein is an about 27.3-kDA cytolytic protein of B. "thuringiensis" or a fragment thereof having high affinity for lipid components of membranes.

CLMS(18) 18. . . . protein is the about 25-kDA Cyt A protein, the protease resistant domain of an about 27.3-kDA cytolytic protein of B. "thuringiensis" subsp. israelensis or morrisoni.

CLMS(19) 19. . . . 14 wherein said insecticidal protein is delivered to the gut epithelium of said target insect in the form of a "chimeric" protein comprising said insecticidal protein or protein domain and said targeting protein.

CLMS(20) 20. The method of claim 19 wherein said "chimeric" protein comprises a crystal protein of Bacillus hurinoiensis (B. "thuringiensis") or a fragment thereof having insecticidal activity and a surface glycoprotein of the extracellular form of a nuclear polyhedrosis. . .

CLMS(21) 21. The method of claim 20 wherein said "chimeric" protein comprises the toxic domain of a B. "thuringiensis" tenebriosis crystal protein and the gp64 glycoprotein of the extracellular form of Autographa californica Nuclear Polyhedrosis Virus (AcNPV).

CLMS(22) 22. The method of claim 19 wherein said "chimeric" protein comprises the toxic domain of a B. "thuringiensis" crystal protein and an about 27.3-kDA cytolytic protein of B. "thuringiensis" or a fragment thereof having high affinity for lipid components of membranes.

CLMS(23) 23. The method of claim 22 wherein said "chimeric" protein comprises the toxic doman of a B. "thuringiensis" crystal protein and the about 25-kDA Cyt A protein, the protease resistant domain of an about 27.3-kDA cytolytic protein of B. "thuringiensis" subsp. israelensis or morrisoni.

US PAT NO: 5,055,294 [IMAGE AVAILABLE] L10: 29 of 31

TITLE: "Chimeric" Bacillus "thuringiensis" crystal protein gene comprising HD-73 and Berliner 1715 "toxin" genes, transformed and expressed in Pseudomonas fluorescens

BSUM(2) The most widely used microbial pesticides are derived from the bacterium Bacillus "thuringiensis". This bacterial agent is used to control a wide range of leaf-eating caterpillars, Japanese beetles and mosquitoes. Bacillus "thuringiensis" produces a proteinaceous paraspore or crystal which is toxic upon ingestion by a susceptible insect host. For example, B. "thuringiensis" var. kurstaki HD-1 produces a crystal called a delta "toxin" which is toxic to the larvae of a number of lepidopteran insects. The cloning and expression of this B.t. crystal. . . Pat. No. 4,467,036 both disclose the expression of B.t. crystal protein in E. coli. In U.S. Pat. No. 4,467,036 B. "thuringiensis" var. kurstaki HD-1 is disclosed as being available from the well-known NRRL culture repository at Peoria, Ill. Its accession number there is NRRL B3792. B. "thuringiensis" var. kurstaki HD-73 is also available from NRRL. Its accession number is NRRL B-4488.

BSUM(5) Specifically, the invention comprises a novel hybrid delta "endotoxin" gene comprising part of the B. "thuringiensis" var. kurstaki strain HD-73 "toxin" gene and part of the "toxin" gene from B. "thuringiensis" var. "thuringiensis" strain Berliner 1715 (DNA 5:305-314, 1986). This hybrid gene was inserted into a suitable transfer vector which was then used. . .

DETD(2) The novel hybrid *toxin* gene of the subject invention comprises part of the B. *thuringiensis* var. kurstaki strain HD-73 *toxin* gene and part of a B. *thuringiensis* var. *thuringiensis* strain Berliner 1715 *toxin* gene. In general, the B.t.k. HD-73 gene portion was initially combined with DNA segments derived from (1) the known E.. . . coli plasmid pBR322, and (2) a DNA segment representing the hybrid Tac promoter. Next, the 3' part of the Berliner *toxin* gene was added followed by a DNA segment conferring the ability to replicate in Pseudomonas from the known plasmid pR01614,

DETD(30) A portion of the B. *thuringiensis* var. kurstaki HD-73 gene including all of the *toxin*-encoding DNA from the starting ATG (i.e., start methionine) to the HindIII site was inserted into the Tac-promoted plasmid pKK223-3 (Pharmacia). This was done by making a blunt *fusion* of this gene just downstream from the ribosome binding site in pKK223-3. This formed plasmid pKK2. Plasmid pKK2 was completely digested with HindIII which cleaves at the ultimate 3' end of the *toxin* gene. The HindIII overhang was made blunt by filling it in with deoxynucleotides using Klenow fragment. Next, PstI linker was. . . by digestion with PstI and closing up of the plasmid with DNA ligase. Then the 3' portion of the Berliner *toxin* sequence (DNA 5:305-314, 1986) was cloned as a SacI to PstI fragment into the modified pKK2/SacI+PstI thus making a recombinant *toxin* gene in a new plasmid named pKK73BB-9. This gene is of Berliner origin for all sequences beyond (3' to) the. . .

DETD(31) Next, pKK73BB-9 was completely digested with PstI, which cleaves at the ultimate 3' end of the new *chimeric* *toxin* gene, and treated with bacterial alkaline phosphatase. The PstI DNA fragment, from plasmid pR01614, which confers the ability to replicate. . .

CLMS(21) 21. Treated, substantially intact unicellular microorganism cells containing an intracellular *toxin*, which *toxin* is a result of expression of a Bacillus *thuringiensis* *toxin* gene toxic to lepidopteran insects which codes for a polypeptide *toxin* having the amino acid sequence shown in FIG. 2, wherein said cells are treated under conditions which prolong the insecticidal. . .

US PAT NO: H 875 [IMAGE AVAILABLE] L10: 30 of 31
TITLE: *Toxin*-encoding nucleic acid fragments derived from a Bacillus *thuringiensis* subsp. israelensis gene

ABSTRACT: Novel . . . encoded thereby, insecticidal compositions containing such proteins, and the use of these proteins in combatting insects, particularly mosquitoes, are described. *Chimeric* genes containing the novel nucleic acid fragments, and microorganisms, tissues, seeds, and plants incorporating the nucleic acid fragments are also. . .

BSUM(4) The spore-forming bacteria Bacillus *thuringiensis* var. israelensis produces a proteinaceous crystalline inclusion which is toxic to the larvae of Mosquito News, 37: 355-358 (1977); de. . . of these polypeptides, and its larvicidal and haemolytic properties have been studied using both purified preparations of the 27 kDa .delta.-*endotoxin* and a 25 kDa segment thereof. See, Davidson et al., Curr. Microbiol., 11: 171-174 (1984); Thomas, W. E. , Ph.D., Thesis, University of Cambridge, "Biochemistry and Mode of Action of the Insecticidal .delta.-endotoxins of Bacillus *thuringiensis*" (1984); Armstrong, et al., J. Bacteriol., 161: 39-46 (1985); Wu et al., FEBS Letts., 190: 232-236 (1985); Lee et al.,. . .

BSUM(5) Using a somewhat different approach to investigate the properties of this polypeptide, the gene encoding the 27 kDa .delta.-*endotoxin* has been cloned in both Escherichia coli (see, Ward et al., FEBS Letts., 175: 377-781 (1984); Waalwijk et al., Nucleic. . . al., J. Mol. Biol., 191: 13-22 (1986)). In E. coli, induction of a high level of wild type 27 kDa .delta.-*endotoxin* expression has been found to have a significant deleterious effect on the growth of that bacterium. It has been postulated that the observed deleterious effect is due to binding of the *toxin* to phosphatidyl choline and phosphatidyl ethanolamine lipid receptors in E. coli cell plasma membranes. See, Ward, E. S., Ph.D. Thesis, University of Chambridge, "Molecular Genetics of an Insecticidal .delta.-*endotoxin* from Bacillus *thuringiensis* var. israelensis" (1988); Thomas, et al., FEBS Letters, 154: 362-368 (1983). In B. subtilis recombinants, the 27 kDa protein accumulates. . . but smaller than, the var. israelensis crystal. These inclusions have been purified and shown to consist entirely of 27 kDa .delta.-*endotoxin*. See, Ward et al., J. Mol. Biol., 191: 1-11 (1986); Ward et al., J. Mol Biol., 191: 13-22 (1986). Testing. . . (1984), Thomas, W. E., Ph.D. Thesis, University of Cambridge, "Biochemistry and Mode of Action of the Insecticidal .delta.-endotoxins of Bacillus *thuringiensis*" (1984), and Armstrong et al., J. Bacteriol., 161: 39-46 (1985), but differ from those of several other groups who did. . .

BSUM(6) The nucleotide sequence of the 27 kDa .delta.-*endotoxin* has been reported in the literature. See, Waalwijk et al., Nucleic Acids Res., 13: 8207-8217 (1985); Ward et al., J. . . . Knowles et al. Biochem. Biophys. Acta., 924: 509-518 (1987) that this protein shares a common cytolytic mechanism with other B. *thuringiensis* .delta.-endotoxine from other serotypes. Commentators in this field have theorized that these .delta.-endotoxins bind to receptors on the membrane, and. . .

BSUM(7) The present invention is based on a more detailed understanding of the interaction of the var. israelensis 27 kDa .delta.-*endotoxin* with target membranes. Through in vitro mutagenesis techniques, specific codon alterations have been directed in the cloned .delta.-*endotoxin* gene. Various of the mutant proteins have been found to possess greater solubility characteristics, less haemolytic activity, and/or greater expression potential in cells containing significant amounts of phosphatidate-type *toxin* receptors, than the protein encoded by the wild type 27 kDa Bacillus *thuringiensis* var. israelensis gene.

BSUM(46) In . . . pertains to nucleic acid fragments coding for insecticidal proteins having greater expression potential in cells containing significant amounts of phosphatidate-type *toxin* receptors than the protein encoded by the wild type 27 kDa Bacillus *thuringiensis* var. israelensis gene.

DETD(13) The phrase "*chimeric* gene" as employed herein refers to a hybrid construct comprising (1) a nucleic acid fragment in accordance with the present. . . from a different source comprises a promoter, although it can also include, for example, nucleic acid fragments from other Bacillus *thuringiensis* *toxin* genes of subspecies israelensis or other subspecies such as aizawai, kurstaki, etc.. Further suitable nucleic acid fragments from different sources. . .

DETD(23) In . . . to nucleic acid fragments coding for insecticidal proteins having a greater expression potential in cells containing significant amounts of phosphatidate-type *toxin* receptors than the protein encoded by the wild type 27 kDa Bacillus *thuringiensis* var. israelensis gene. Preferably, the cells containing significant amounts of phosphatidate-type *toxin* receptors are E. coli cells. This discovery permits effective production of the insecticidal protein in a number of cells, including. . .

DETD(55) The strains of E. coli utilized as cloning hosts for both the wild type 27000 Da .delta.-*endotoxin* Bacillus *thuringiensis* var. israelensis gene and the mutant derivatives were E. coli TG1 (K12, .alpha.(lac-pro), supE, thi, hsdD5/F'traD36, proA+B+, lacI.sup.q, lacZ.DELTA.M15), available. . . School of Medicine, Philadelphia, Pa. 19140, were also used as cloning hosts for preparation of the wild type 27000 Da .delta.-*endotoxin* and the mutant derivatives.

DETD(62) Site-Directed Mutagenesis of the Bacillus *thuringiensis* subsp. israelensis 27 kDa .delta.-*Endotoxin* Gene and Expression of the Resultant Mutated Nucleic Acid Fragments

DETD(63) The . . . et al., Nature (London), 299: 756-758 (1982). A 790 bp or 425 bp PstI fragment, containing a portion of the .delta.-*endotoxin* gene and either 5' or 3' flanking regions were generated using a PstI site in the 27 kDa .delta.-*endotoxin* Bacillus *thuringiensis* var. israelensis genome and a PstI site in the polylinker of the cloning vector pUC12 (described by Messing, J. Meths.. . . into the PstI site of phages M13tg130 in both orientations, and recombinants producing single stranded DNA (ssDNA) containing the non-coding .delta.-*endotoxin* strand was used as a template to generate mutants.

DETD(69) Specifically, . . . et al., J. Mol. Biol., 191: 1-11 (1986); Ward et al., J. Mol. Biol., 191: 13-22 (1986)) resulting in a *chimeric* plasmid consisting of pUC12 (described in Messing, Meths. Enzymol., 101: 20-78 (1983) and found in Camtaq 11 and Cambtaq 6),. . . to herein inh an abbreviated fashion using, for example, the designation Ala45 to indicate the presence of a 27 kDa .delta.-*endotoxin* gene containing nucleotide change(s) that resulted in an amino acid change at position 45 from glutamic acid in the wild. . .

DETD(71) Recombinant B. subtilis cells harbouring *chimeric* plasmids containing the mutant .delta.-*endotoxin* genes were grown as previously described. See, Ward et al., J. Mol. Biol., 191: 1-11 (1986); Ward et al., J.. . .

DETD(79) Recombinant E. coli cells harbouring *chimeric* plasmids containing the mutation from Glu45 to Ala45 were cultured in the presence of 0.5 mM IPTG as previously described in Ward, E. S., Ph.D. Thesis, University of Cambridge, "Molecular Genetics of an Insecticidal .delta.-*endotoxin* from Bacillus *thuringiensis* var. israelenis" (1988), and their growth patterns observed and compared to the E. coli cells harbouring the wild type gene.. . .

DETD(81) A. . . binding to phosphatidyl choline liposomes (PC Binding) was also made, since it has been postulated that binding of the protein *toxin* to phosphatidyl choline present in the cell plasma membrane is one important factor in the *toxin*'s cytolytic process. The PC binding determination was made using the procedures reported in Ellar et al., Biochemistry, Genetics and Mode of Action of Bacillus "Thuringiensis" .delta.-endotoxins, in "Molecular Biology of Microbial Differentiation", pp. 230-240 (American Society for Microbiology, Washington, D.C., 1985). It should be noted. . .

CLMS(11) 11. A microorganism selected from the group consisting of Bacillus megaterium, Bacillus subtilis and Bacillus *thuringiensis* containing a nucleic acid fragment according to claim 1.

US PAT NO: 4,945,057 [IMAGE AVAILABLE] L10: 31 of 31
TITLE: Monoclonal antibodies to crystal protein of Bacillus *thuringiensis* subspecies israelensis

ABSTRACT: Murine hybridomas are disclosed which were constructed by *fusing* spleen cells from BALB/c mice immunized with soluble crystal protein from Bacillus *thuringiensis* subsp. israelensis (B.t.i.) to the murine myeloma cell line SP2/0-AG14. An ELISA (enzyme-linked immunosorbent assay) method for detection of antibodies. . . culture supernatant fluid indicated production of monoclonal IgG3 antibodies, specific for the 68,000 dalton protein presumed to be the insecticidal delta-*endotoxin* of B.t.i.

BSUM(5) Bacillus *thuringiensis* is an aerobic, endospore-forming, Gram positive bacterium which forms a proteinaceous crystalline inclusion during sporulation and is pathogenic to certain insects. Monoclonal antibodies to Bacillus *thuringiensis* have been reported for crystal protein only of subsp. *thuringiensis* and kurstaki (Huber-Lukac et al., 1982, 1983, 1986). The crystal protein of B. *thuringiensis* subsp. israelensis is larvicidal to several families of Dipteran insects, including mosquitoes, black flies, midges, and horn flies (Temeyer 1984). Crystals prepared from B. *thuringiensis* subsp. israelensis contain several proteins and there is some controversy over which of these component proteins represents the insecticidal *toxin* (delta-*endotoxin*) active in vivo. Molecular biological techniques are capable of resolving this controversy, but are complicated by potential proteolysis of crystal. . .

DETD(33) Monoclonal antibodies to B. *thuringiensis* subsp. *thuringiensis* delta-*endotoxin* were constructed by Huber-Lukac et al. (1982). They reported that all of their monoclonal antibodies cross-reacted with protoxin in an. . . crystal protein formation, crystallization or solubilization. The availability of monoclonal antibodies will aid investigations of the molecular biology of Bacillus *thuringiensis* subsp. israelensis by enabling both immunochemical studies of crystal proteins and molecular cloning of specific crystal protein genes.

CLMS(1) We . . . or a subclone thereof which produces and secretes monoclonal antibody that specifically binds to the 68K crystal protein of Bacillus *thuringiensis* subsp. israelensis.

L11 1. 5,885,603, Mar. 23, 1999, Insecticidal matrix and process for preparation thereof; Jeffrey D. Fowler, et al., 424/405, 408, 409, 417, 421, 485, 491, 496, 497, 499, 500, 501 [IMAGE AVAILABLE] APPL-NO: 08/908,290 DATE FILED: Aug. 7, 1997 REL-US-DATA: Continuation of Ser. No. 654,512, Jun. 12, 1996, Pat. No. 5,851,545.

2. 5,851,545, Dec. 22, 1998, Insecticidal matrix and process for preparation thereof; Jeffrey D. Fowler, et al., 424/405, 93.3, 409, 461, 486, 487 [IMAGE AVAILABLE] APPL-NO: 08/654,512 DATE FILED: Jun. 12, 1996

3. 5,827,515, Oct. 27, 1998, Bacillus thuringiensis sporulation gene; Mitra Shahabi Reynoso, et al., 424/93.2, 93.461, 405; 435/6, 69.1, 71.3, 242, 252.31, 320.1, 471, 490; 536/23.7, 23.71, 24.3 [IMAGE AVAILABLE]

4. 5,736,131, Apr. 7, 1998, Hybrid toxin; Hendrik Jan Bosch, et al., 800/300; 424/93.1, 93.2, 93.461; 435/69.7, 252.3, 252.31, 254.11, 320.1; 514/2, 12; 530/350; 536/23.4, 23.71 [IMAGE AVAILABLE] APPL-NO 08/671,947 DATE FILED: Jul. 15, 1996

US PAT NO: 5,885,603 [IMAGE AVAILABLE] L11: 1 of 4
DETD(5) The . . . in the invention. However, while not meant to limit the invention in any manner, preferred toxin proteins include CryIA, CryIB, *CryIC*, CryID, CryIE, CryIF, *CryIG*, CryII, CryIII, CryIV, CryIH, CryV, CtyA, CytB and any variants, mixtures or parts thereof. Particularly preferred toxins include CryIC, CryIA(a),. . .

US PAT NO: 5,851,545 [IMAGE AVAILABLE] L11: 2 of 4
DETD(5) The . . . in the invention. However, while not meant to limit the invention in any manner, preferred toxin proteins include CryIA, CryIB, *CryIC*, CryID, CryIE, CryIF, *CryIG*, CryII, CryIII, CryIV, CryIH, CryV, CtyA, CytB and any variants, mixtures or parts thereof. Particularly preferred toxins include CryIC, CryIA(a),. . .

US PAT NO: 5,827,515 [IMAGE AVAILABLE] L11: 3 of 4
CLMS(6) 6. . . . to claim 4 wherein said crystal protein encoding sequence is selected from the group consisting of cryIA(a), cryIA(b), cryIA(c), cryIB, *cryIC*, cryID, cryIE, cryIF, *cryIG*, cryIH, cryIIA, cryIIb, cryIIIA, cryIIIB, cryIIIC, cryIVA, cryIVB, cryIVC, cryIVD, cryV genes, mixtures thereof and sequences constructed from parts of. . .

CLMS(19) 19. . . . to claim 18 wherein said crystal protein encoding gene is selected from the group consisting of cryIA(a), cryIA(b), cryIA(c), cryIB, *cryIC*, cryID, cryIE, cryIF, *cryIG*, cryIH, cryIIA, cryIIb, cryIIIA, cryIIIB, cryIIIC, cryIVA, cryIVB, cryIVC, cryIVD, cryV genes, mixtures thereof and sequences constructed from parts of. . .

L12 1. 5,889,174, Mar. 30, 1999, Nucleotide sequences encoding pesticidal proteins; Gregory W. Warren, et al., 536/23.71; 435/69.1; 536/23.7 [IMAGE AVAILABLE]

2. 5,888,801, Mar. 30, 1999, Pesticidal strains of bacillus; Gregory W. Warren, et al., 435/252.5; 424/93.46 [IMAGE AVAILABLE]

3. 5,885,603, Mar. 23, 1999, Insecticidal matrix and process for preparation thereof; Jeffrey D. Fowler, et al., 424/405, 408, 409, 417, 421, 485, 491, 496, 497, 499, 500, 501 [IMAGE AVAILABLE]

4. 5,885,571, Mar. 23, 1999, Bacillus thuringiensis strains and their insecticidal proteins; Bart Lambert, et al., 424/93.461, 93.2; 435/71.3, 252.3, 252.31, 252.5, 320.1; 514/12; 536/23.1, 23.71 [IMAGE AVAILABLE]

5. 5,872,212, Feb. 16, 1999, Pesticidal proteins and strains; Gregory W. Warren, et al., 530/350, 825 [IMAGE AVAILABLE]

6. 5,866,326, Feb. 2, 1999, Method for isolating vegetative insecticidal protein genes; Gregory W. Warren, et al., 435/6, 91.1; 536/25.4 [IMAGE AVAILABLE]

7. 5,861,543, Jan. 19, 1999, Bacillus thuringiensis strains and their insecticidal proteins; Bart Lambert, et al., 800/279; 435/320.1, 419, 424, 430; 536/23.71; 800/302, 320.1 [IMAGE AVAILABLE]

8. 5,851,545, Dec. 22, 1998, Insecticidal matrix and process for preparation thereof; Jeffrey D. Fowler, et al., 424/405, 93.3, 409, 461, 486, 487 [IMAGE AVAILABLE]

9. 5,849,870, Dec. 15, 1998, Pesticidal proteins and strains; Gregory W. Warren, et al., 530/350; 435/252.31, 252.5, 320.1; 536/23.1, 23.7, 23.71 [IMAGE AVAILABLE]

10. 5,840,868, Nov. 24, 1998, Pesticidal proteins and strains; Gregory W. Warren, et al., 536/23.1; 435/6, 320.1; 530/350; 536/24.1 [IMAGE AVAILABLE]

11. 5,827,515, Oct. 27, 1998, Bacillus thuringiensis sporulation gene; Mitra Shahabi Reynoso, et al., 424/93.2, 93.461, 405; 435/6, 69.1, 71.3, 242, 252.31, 320.1, 471, 490; 536/23.7, 23.71, 24.3 [IMAGE AVAILABLE]

12. 5,770,696, Jun. 23, 1998, Auxiliary proteins for enhancing the insecticidal activity of pesticidal proteins; Gregory W. Warren, et al., 530/350, 825; 536/23.1, 23.7, 23.71 [IMAGE AVAILABLE]

L3 1. 5,885,603, Mar. 23, 1999, Insecticidal matrix and process for preparation thereof; Jeffrey D. Fowler, et al., 424/405, 408, 409, 417, 421, 485, 491, 496, 497, 499, 500, 501 [IMAGE AVAILABLE]

2. 5,851,545, Dec. 22, 1998, Insecticidal matrix and process for preparation thereof; Jeffrey D. Fowler, et al., 424/405, 93.3, 409, 461, 486, 487 [IMAGE AVAILABLE]

US PAT NO: 5,885,603 [IMAGE AVAILABLE] L13: 1 of 2
DETD(6) Recombinant . . . of toxin proteins a particular Bacillus strain produces and the use of protein design to create a gene expressing a *fusion* or hybrid protein. An example of a hybrid gene is G27, containing fragments of different Cry proteins and specifically *CryIE* and *CryIC*. This protein is further described in Bosch et al., Biotechnology 12:915-918 (1994) which is hereby incorporated by reference. Those skilled. . .

L15 . 5,843,744, Dec. 1, 1998, Bacillus thuringiensis Tn5401 proteins; James A. Baum, 435/320.1, 252.31 [IMAGE AVAILABLE] APPL-NO: 08/266,408 DATE FILED: Jun. 24, 1994 REL-US-DATA: Continuation-in-part of Ser. No. 89,986, Jul. 8, 1993, Pat. No. 5,441,884.

2. 5,840,554, Nov. 24, 1998, .beta.-Endotoxin expression in pseudomonas fluorescens; Mark Thompson, et al., 435/471; 424/405, 538; 435/69.7, 252.34, 320.1, 480; 514/2; 530/350; 536/23.4, 23.71 [IMAGE AVAILABLE] APPL-NO: 08/639,923 DATE FILED: Apr. 24, 1996 REL-US-DATA: Division of Ser. No. 239,476, May 6, 1994, Pat. No. 5,527,883.

3. 5,827,514, Oct. 27, 1998, Pesticidal compositions; Gregory A. Bradfisch, et al., 424/93.2, 93.1, 93.3; 435/69.1, 69.7, 252.3, 410, 418, 419 [IMAGE AVAILABLE] APPL-NO: 08/598,305 DATE FILED: Feb. 8, 1996 REL-US-DATA: Continuation of Ser. No. 349,867, Dec. 6, 1994, Pat. No. 5,508,264.

4. 5,650,308, Jul. 22, 1997, Recombinant Bacillus thuringiensis strain construction method; James A. Baum, 435/485, 252.31, 320.1 [IMAGE AVAILABLE] APPL-NO: 08/478,585 DATE FILED: Jun. 7, 1995 REL-US-DATA: Division of Ser. No. 89,986, Jul. 8, 1993, Pat. No.5,441,884.

5. 5,593,881, Jan. 14, 1997, Bacillus thuringiensis delta-endotoxin; Mark Thompson, et al., 435/418, 252.3, 320.1; 536/23.71 [IMAGE AVAILABLE] APPL-NO: 08/239,474 DATE FILED: May 6, 1994

6. 5,527,883, Jun. 18, 1996, Delta-endotoxin expression in pseudomonas fluorescens; Mark Thompson, et al., 530/350; 435/252.34, 320.1; 536/23.4, 23.71 [IMAGE AVAILABLE] APPL-NO: 08/239,476 DATE FILED: May 6, 1994

7. 5,508,264, Apr. 16, 1996, Pesticidal compositions; Gregory A. Bradfisch, et al., 514/12; 530/350 [IMAGE AVAILABLE] APPL-NO: 08/349,867 DATE FILED Dec. 6, 1994

8. 5,441,884, Aug. 15, 1995, Bacillus thuringiensis transposon TN5401; James A. Baum, 435/252.31; 424/93.2; 435/252.3, 252.33, 320.1; 536/23.1, 23.2, 23.7, 24.1 [IMAGE AVAILABLE] APPL-NO: 08/089,986 DATE FILED: Jul. 8, 1993

US PAT NO: 5,843,744 [IMAGE AVAILABLE] L15: 1 of 8
DETD(98) The . . . the B.t. origin of replication ori43, was manipulated to replace ori43 with a cryl-type B.t. protein toxin gene, specifically a *crylC*-*crylA*(c) *fusion* gene. The choice of the specific B.t. toxin gene for insertion into p85 is not critical; any insecticidal protein toxin. . .

DETD(115) Plasmid shuttle vector pEG928.9, containing a cryl-type gene (a *crylC*-*crylA*(c) *fusion* gene), a B.t. origin of replication region (ori43.9, a high copy number mutant of ori143, derived from a 43-MDa B.t.. . .

DETD(120) The . . . plasmid pEG928.9.DELTA.. Plasmid pEG928.9.DELTA.is an 8.0 kb recombinant plasmid that contains the ori43.9 origin of replication functional in B.t., the *crylC*-*crylA*(c) B.t. protein toxin *fusion* gene, and a single copy of the internal resolution site, derived from the site-specific recombination event.

DETD(125) B.t. . . . no DNA not native to B.t., is -insecticidal to a wide spectrum of lepidopteran insects and, because of the additional *crylC*-*crylA*(c) *fusion* gene on its recombinant plasmid pEG928.9.DELTA., is designed to exhibit improved insecticidal activity against Spodoptera exigua (beet armyworm) and Spodoptera. . .

DETD(128) The . . . in FIG. 11. Plasmid shuttle vector pEG931 is similar to plasmid pEG928.9 except that (i) a crylC gene replaces the *crylC*-*crylA*(c) *fusion* gene of pEG928.9, (ii) it contains a transcription terminator downstream of the crylC gene, and (iii) the B.t. origin of. . .

US PAT NO: 5,840,554 [IMAGE AVAILABLE] L15: 2 of 8
BSUM(8) *Chimeric* proteins joined within the toxin domains have been reported between *CrylC* and *CrylA*(b) (Honee, G., D. Convents, J. Van Rie, S. Jansens, M. Perferoen, B. Visser [1991] Mol. Microbiol. 5:2799-2806); however, the activity of these *chimeric* proteins was either much less, or undetectable, when compared to CrylC on a relevant insect.

BSUM(9) Honee et al. (Honee, G., W. Vriezen, B. Visser [1990] Appl. Environ. Microbiol. 56:823-825) also reported making a *chimeric* *fusion* protein by linking tandem toxin domains of *CrylC* and *CrylA*(b). The resulting protein had an increased spectrum of activity equivalent to the combined activities of the individual toxins; however, the activity of the *chimeric* was not increased toward any one of the target insects.

US PAT NO: 5,827,514 [IMAGE AVAILABLE] L15: 3 of 8
BSUM(9) *Chimeric* proteins joined within the toxin domains have been reported between *CrylC* and *CrylA*(b) (Honee, G., D. Convents, J. Van Rie, S. Jansens, M. Perferoen, B. Visser [1991] Mol. Microbiol. 5:2799-2806); however, the activity of these *chimeric* proteins was either much less, or undetectable, when compared to CrylC on a relevant insect.

BSUM(10) Honee et al. (Honee, G., W. Vriezen, B. Visser [1990] Appl. Environ. Microbiol. 56:823-825) also reported making a *chimeric* *fusion* protein by linking tandem toxin domains of *CrylC* and *CrylA*(b). The resulting protein had an increased spectrum of activity equivalent to the combined activities of the individual toxins; however, the activity of the *chimeric* was not increased toward any one of the target insects.

US PAT NO: 5,650,308 [IMAGE AVAILABLE] L15: 4 of 8
DETD(97) The . . . the B.t. origin of replication ori43, was manipulated to replace ori43 with a cryl-type B.t. protein toxin gene, specifically a *crylC*-*crylA*(c) *fusion* gene. The choice of the specific B.t. toxin gene for insertion into p85 is not critical; any insecticidal protein toxin. . .

DETD(114) Plasmid shuttle vector pEG928.9, containing a cryl-type gene (a *crylC*-*crylA*(c) *fusion* gene), a B.t. origin of replication region (ori43.9, a high copy number mutant of ori43, derived from a 43-MDa B.t.. . .

DETD(119) The . . . pEG928.9.DELTA.. Plasmid pEG928.9.DELTA. is a 8.0 mDa recombinant plasmid that contains the ori43.9 origin of replication functional in B.t., the *crylC*-*crylA*(c) B.t. protein toxin *fusion* gene, and a single copy of the internal resolution site, derived from the site-specific recombination event.

DETD(124) B.t. . . . no DNA not native to B.t., is insecticidal to a wide spectrum of lepidopteran insects and, because of the additional *crylC*-*crylA*(c) *fusion* gene on its recombinant plasmid pEG928.9.DELTA., is designed to exhibit improved insecticidal activity against Spodoptera exigua (beet armyworm) and Spodoptera. . .

DETD(127) The . . . in FIG. 11. Plasmid shuttle vector pEG931 is similar to plasmid pEG928.9 except that (i) a crylC gene replaces the *crylC*-*crylA*(c) *fusion* gene of pEG928.9, (ii) it contains a transcription terminator downstream of the crylC gene, and (iii) the B.t. origin of.

US PAT NO: 5,593,881 [IMAGE AVAILABLE] L15: 5 of 8
DETD(8) A . . . to some extent in length and the precise location of the transition from toxin portion to protoxin portion. Typically, the *crylA*(b) and *crylC* toxins will be about 1150 to about 1200 amino acids in length. The transition from toxin portion to protoxin portion will typically occur at between about 50% to about 60% of the full length toxin. The *chimeric* toxin of the subject invention will include the full expanse of this core N-terminal toxin portion. Thus, the *chimeric* toxin will comprise at least about 50% of the full length B.t. toxin. This will typically be at least about. . . is the last about 100 to 150 amino acids of this portion which are most critical to include in the *chimeric* toxin of the subject invention. In a *chimeric* toxin specifically exemplified herein, at least amino acids 1085 to the C-terminus of the crylA(b) molecule are utilized. Thus, it. . . the overall B.t. protein which should comprise heterologous DNA (compared to the crylF core N-terminal toxin portion) included in the *chimeric* toxin of the subject invention. Thus, a preferred embodiment of the subject invention is a *chimeric* B.t. toxin of about 1150 to about 1200 amino acids in length, wherein the *chimeric* toxin comprises a crylC core N-terminal toxin portion of at least about 50 to 60% of a full crylC molecule, but no more than about 90 to 95% of the full molecule. The *chimeric* toxin further comprises a crylA(b) protoxin C-terminal portion which comprises at least about 5 to 10% of the crylA(b) molecule. The transition from *crylC* to *crylA*(b) sequence thus occurs within the protoxin segment (or at the junction of the toxin and protoxin segments) between about 50% and about 95% of the way through the molecule. In the specific example provided herein, the transition from the *crylC* sequence to the *crylA*(b) sequence occurs prior to amino acid 1085 of the *chimeric* toxin.

US PAT NO: 5,527,883 [IMAGE AVAILABLE] L15: 6 of 8
BSUM(8) *Chimeric* proteins joined within the toxin domains have been reported between *CrylC* and *CrylA*(b) (Honee, G., D. Convents, J. Van Rie, S. Jansens, M. Perferoen, B. [redacted] [1991] Mol. Microbiol. 5:2799-2806); however, the activity of these *chimeric* proteins was e[redacted] much less, or undetectable, when compared to CrylC on a relevant insect.

BSUM(9) Honee et al. (Honee, G., W. Vriezen, B. Visser [1990] Appl. Environ. Microbiol. 56:823-825) also reported making a *chimeric* *fusion* protein by linking tandem toxin domains of *CrylC* and *CrylA*(b). The resulting protein had an increased spectrum of activity equivalent to the combined activities of the individual toxins; however, the activity of the *chimeric* was not increased toward any one of the target insects.

US PAT NO: 5,508,264 [IMAGE AVAILABLE] L15: 7 of 8
BSUM(9) *Chimeric* proteins joined within the toxin domains have been reported between *CrylC* and *CrylA*(b) (Honee, G., D. Convents, J. Van Rie, S. Jansens, M. Perferoen, B. Visser [1991] Mol. Microbiol. 5:2799-2806); however, the activity of these *chimeric* proteins was either much less, or undetectable, when compared to CrylC on a relevant insect.

BSUM(10) Honee et al. (Honee, G., W. Vriezen, B. Visser [1990] Appl. Environ. Microbiol. 56:823-825) also reported making a *chimeric* *fusion* protein by linking tandem toxin domains of *CrylC* and *CrylA*(b). The resulting protein had an increased spectrum of activity equivalent to the combined activities of the individual toxins; however, the activity of the *chimeric* was not increased toward any one of the target insects.

US PAT NO: 5,441,884 [IMAGE AVAILABLE] L15: 8 of 8
DETD(97) The . . . the B.t. origin of replication ori43, was manipulated to replace ori43 with a cryl-type B.t. protein toxin gene, specifically a *crylC*-*crylA*(c) *fusion* gene. The choic[redacted] the specific B.t. toxin gene for insertion into p85 is not critical; any insecticidal protein toxin[redacted]

DETD(114) Plasmid shuttle vector pEG928.9, containing a cryl-type gene (a *crylC*-*crylA*(c) *fusion* gene), a B.t. origin of replication region (ori43.9, a high copy number mutant of ori43, derived from a 43-MDa B.t.. . .

DETD(119) The . . . pEG928.9.DELTA.. Plasmid pEG928.9.DELTA. is a 8.0 mDa recombinant plasmid that contains the ori43.9 origin of replication functional in B.t., the *crylC*-*crylA*(c) B.t. protein toxin *fusion* gene, and a single copy of the internal resolution site, derived from the site-specific recombination event.

DETD(124) B.t. . . . no DNA not native to B.t., is insecticidal to a wide spectrum of lepidopteran insects and, because of the additional *crylC*-*crylA*(c) *fusion* gene on its recombinant plasmid pEG928.9.DELTA., is designed to exhibit improved insecticidal activity against Spodoptera exigua (beet armyworm) and Spodoptera. . .

DETD(127) The . . . in FIG. 11. Plasmid shuttle vector pEG931 is similar to plasmid pEG928.9 except that (i) a crylC gene replaces the *crylC*-*crylA*(c) *fusion* gene of pEG928.9, (ii) it contains a transcription terminator downstream of the crylC gene, and (iii) the B.t. origin of. . .

L17 1. 5,885,603, Mar. 23, 1999, Insecticidal matrix and process for preparation thereof; Jeffrey D. Fowler, et al., 424/405, 408, 409, 417, 421, 485, 491, 496, 497, 499, 500, 501 [IMAGE AVAILABLE] APPL-NO 08/908,290 DATE FILED: Aug. 7, 1997 REL-US-DATA: Continuation of Ser. No. 654,512, Jun. 12, 1996, Pat. No. 5,851,545.

2. 5,851,545, Dec. 22, 1998, Insecticidal matrix and process for preparation thereof; Jeffrey D. Fowler, et al., 424/405, 93.3, 409, 461, 486, 487 [IMAGE AVAILABLE] APPL-NO 08/654,512 DATE FILED: Jun. 12, 1996

3. 5,659,123, Aug. 19, 1997, Diabrotica toxins; Jeroen Van Rie, et al., 800/302; 514/12; 536/23.71; 800/320.1 [IMAGE AVAILABLE] APPL-NO: 08/295,060 DATE FILED: Aug. 26, 1994

4. 5,628,995, May 13, 1997, Control of Ostrinia; Marnix Peferoen, et al., 800/279; 424/93.2, 93.21; 514/12; 800/294 [IMAGE AVAILABLE] APPL-NO: 08/377,690 DATE FILED: Jan. 25, 1995 FRN-PR. NO: 92402307 FRN FILED: Aug. 19, 1992 FRN-PR. CO: European Patent Office REL-US-DATA: Continuation of Ser. No. 164,781, Dec. 10, 1993, abandoned, which is a continuation of Ser. No. 938,362, Aug. 31, 1992, abandoned.

5. 5,530,197, Jun. 25, 1996, Control of ostrinia; Marnix Peferoen, et al., 800/279; 424/93.2, 93.21; 514/12; 800/288, 302 [IMAGE AVAILABLE] APPL-NO: 08/463,513 DATE FILED: Jun. 5, 1995 FRN-PR. NO: 92402307 FRN FILED: Aug. 19, 1992 FRN-PR. CO: European Patent Office REL-US-DATA: Division of Ser. No. 377,690, Jan. 25, 1995, which is a continuation of Ser. No. 164,781, Dec. 10, 1993, abandoned, which is a continuation of Ser. No. 938,362, Aug. 31, 1992, abandoned.

US PAT NO: 5,885,603 [IMAGE AVAILABLE] L17: 1 of 5
DETD(5) The . . . in the invention. However, while not meant to limit the invention in any manner, preferred toxin proteins include CrylA, CrylB, *CryIC*, CryID, *CryIE*, CryIF, CryIG, Cryli, CryIII, CrylV, CryIH, CryV, CtyA, CytB and any variants, mixtures or parts thereof. Particularly preferred toxins include. . .

DETD(6) Recombinant . . . proteins a particular Bacillus strain produces and the use of protein design to create a gene expressing a fusion or *hybrid* protein. An example of a *hybrid* gene is G27, containing fragments of different Cry proteins and specifically *CrylE* and *CrylC*. This protein is further described in Bosch et al., Biotechnology 12:915-918 (1994) which is hereby incorporated by reference. Those skilled in the art are aware of other *hybrid* genes and the above example is not meant to limit the invention in any manner.

DETD(21) In . . . active toxin selected from the group consisting of CryIC, CrylA(a), CrylA(b), Cry IIA, CrylA(c) and fragments and mixtures thereof including *hybrid* proteins.

US PAT NO: 5,851,545 [IMAGE AVAILABLE] L17: 2 of 5
DETD(5) The . . . in the invention. However, while not meant to limit the invention in any manner, preferred toxin proteins include CrylA, CrylB, *CryIC*, CryID, *CryIE*, CryIF, CryIG, Cryli, CryIII, CrylV, CryIH, CryV, CtyA, CytB and any variants, mixtures or parts thereof. Particularly preferred toxins include. . .

DETD(6) Recombinant . . . proteins a particular Bacillus strain produces and the use of protein design to create a gene expressing a fusion or *hybrid* protein. An example of a *hybrid* gene is G27, containing fragments of different Cry proteins and specifically *CrylE* and *CrylC*. This protein is further described in Bosch et al., Biotechnology 12:915-918 (1994) which is hereby incorporated by reference. Those skilled in the art are aware of other *hybrid* genes and the above example is not meant to limit the invention in any manner.

DETD(21) In . . . active toxin selected from the group consisting of CryIC, CrylA(a), CrylA(b), Cry IIA, CrylA(c) and fragments and mixtures thereof including *hybrid* proteins.

US PAT NO: 5,659,123 [IMAGE AVAILABLE] L17: 3 of 5
BSUM(14) Different sets of *hybrid* ICP genes have been constructed through exchange of gene fragments between ICP genes, encoding ICPs with different insect specificities. The *hybrid* ICPs were tested in bioassays in order to located the specificity-determining region in the parental ICPs (Ge et al., 1989, . . . positions in the CrylIIA structure, and these fall mainly in domain II (Li et al., 1991, supra). From studies with *hybrid* CrylA proteins, Lee et al. (1992, J. Biol. Chem., 267, 3115-3121) concluded that the B. mori receptor-binding region on the. . . toxin could not be excluded (Schnepf et al., 1990, supra; Ge et al., 1991, supra). Furthermore, a recent study using *hybrid* ICPs constructed by exchanging gene fragments between *crylC* and *crylE*, has indicated that domain II of CrylC is not sufficient to confer the high activity of this protein towards Spodoptera. . .

DETD(4) *A . . . protein, as used herein, also include proteins containing the specificity- and toxicity-determining region of the CryII protein, e.g. in a *hybrid* with another protein, such as another Bt ICP, provided the CryII toxicity is substantially retained therein. A CryII protein, as. . .

DETD(5) Following . . . modified CrylIA proteins having altered toxicity to Diabrotica virgifera virgifera, as shown in Table 1. Modified CryIII proteins also include *hybrid* proteins made by transferring a functional part of a modified CryIII protein to another Bt ICP protein, such as a. . .

DETD(6) The . . . the protein, that can be transferred or added to another protein, such as another Bt ICP, for providing a new *hybrid* protein with at least one functional characteristic (e.g., the binding, specificity and/or toxicity characteristics) of the modified CryIII toxin (Ge et al., 1991, supra), that is different from that of the native CryIII protein. Such a *hybrid* protein can have an enlarged host range and/or an improved toxicity. For example, domain II, preferably the regions protruding from. . .

DETD(22) Optionally, to locate the region involved in receptor binding/specificity, either *hybrid* crystal proteins are constructed by exchanging structural domains between the crystal proteins (a); or homolog scanning mutagenesis is performed: exchange. . .

DETD(23) To . . . fragments corresponding to structural domains by splice overlap extension using PCR (Horton et al., 1989, Gene 77, 61-68). Following construction, *hybrid* or mutant genes are then expressed in E. coli or crystal minus B. thuringiensis strains. The mutant or *hybrid* proteins are then tested in toxicity assays on the target insect. By comparing the toxicity of the parental and *hybrid* proteins and considering the sequences of the *hybrid* proteins, the region(s) which are responsible for the higher activity of one of the ICPs are located. Since in general. . .

DETD(24) Knowledge . . . Indeed, the exchange of gene fragments corresponding to such elements is likely to increase the chances of obtaining structurally stable *hybrid* proteins. Gene fragments can, however, be exchanged between ICP genes without knowledge of the location of secondary structural elements.

DETD(39) The chimeric modified cryIII gene, or its insecticidally effective gene part, can optionally be inserted in the plant genome as a *hybrid* gene (EP 0 193 259; Vaeck et al., 1987, supra) under the control of the same promoter as the coding. . .

US PAT NO: 5,628,995 [IMAGE AVAILABLE] L17: 4 of 5
BSUM(15) The . . . has described the insecticidal activity of the following ICPs against various insects, including O. nubilalis: CrylAa, CrylAb, CrylAc, CrylB, CryID, *CryIC* and *CrylE*, and PCT publication WO 92/09696 also has described the insecticidal activity of the crylAb and crylB genes against O. nubilalis.

BSUM(48) It . . . neogene (Reiss et al, 1984; EP 242 236), coding for kanamycin resistance. The transformed cells can be provided with a *hybrid* gene, containing the cry gene(s) and the marker gene under the control of the same promoter. This *hybrid* gene will be expressed in the transformed cells as a fusion protein (Vaeck et al, 1987). Also *hybrid* genes, comprising the active fragments of both the crylB and the crylAb or crylAc genes, can be constructed as described.

US PAT NO: 5,530,197 [IMAGE AVAILABLE] L17: 5 of 5
BSUM(15) The . . . has described the insecticidal activity of the following ICPs against various insects, including O. nubilalis: CrylAa, CrylAb, CrylAc, CrylB, CryID, *CryIC* and *CrylE*, and PCT publication WO 92/09696 also has described the insecticidal activity of the crylAb and crylB genes against O. nubilalis.

L18 1. 5,885,603, Mar. 23, 1999, Insecticidal matrix and process for preparation thereof; Jeffrey D. Fowler, et al., 424/405, 408, 409, 417, 421, 485, 491, 496, 497, 499, 500, 501 [IMAGE AVAILABLE] APPL-NO 08/908,290 DATE FILED: Aug. 7, 1997 REL-US-DATA: Continuation of Ser. No. 654,512, Jun. 12, 1996, Pat. No. 5,851,545.

2. 5,851,545, Dec. 22, 1998, Insecticidal matrix and process for preparation thereof; Jeffrey D. Fowler, et al., 424/405, 93.3, 409, 461, 486, 487 [IMAGE AVAILABLE] APPL-NO: 08/654,512 DATE FILED: Jun. 12, 1996

3. 5,804,393, Sep. 8, 1998, Antibodies directed to the binding proteins of Bacillus thuringiensis and their use; Martin Geiser, et al., 435/7.2, 7.32, 7.92, 7.93, 975; 436/501, 503, 547, 548, 808; 530/387.1, 387.2, 388.22, 389.1 [IMAGE AVAILABLE] APPL-NO: 08/922,254 DATE FILED: Sep. 2, 1997 FRN-PR. NO: 2231/91 FRN FILED: Jul. 25, 1991 FRN-PR. CO: Switzerland FRN-PR. NO: 2517/91 FRN FILED: Aug. 25, 1991 FRN-PR. CO: Switzerland REL-US-DATA: Continuation of Ser. No. 754,334, Nov. 22, 1996, abandoned, which is a continuation of Ser. No. 317,000, Oct. 3, 1994, abandoned, which is a continuation of Ser. No. 918,543, Jul. 21, 1992, abandoned.

4. 5,702,703, Dec. 30, 1997, Bacillus thuringiensis toxin enhancer; H. Ernest Schnepf, et al., 424/93.461; 71/1, 6; 424/93.46; 435/832, 834; 530/350, 825 [IMAGE AVAILABLE] APPL-NO: 08/340,563 DATE FILED: Nov. 16, 1994p

US PAT NO: 5,885,603 [IMAGE AVAILABLE] L18: 1 of 4
DETD(5) The . . . be used in the invention. However, while not meant to limit the invention in any manner, preferred toxin proteins include *CrylA*, CrylB, *CryIC*, CryID, CryIE, CryIF, CryIG, CryII, CryIII, CryIV, CryIH, CryV, CtyA, CytB and any variants, mixtures or parts thereof. Particularly preferred toxins include *CryIC*, *CryIA*(a), *CryIA*(b), CrylA(c), CryIE, CryIIA and variants, mixtures and parts thereof.

DETD(6) Recombinant . . . proteins a particular Bacillus strain produces and the use of protein design to create a gene expressing a fusion or *hybrid* protein. An example of a *hybrid* gene is G27, containing fragments of different Cry proteins and specifically CrylE and CrylC. This protein is further described in. . . et al., Biotechnology 12:915-918 (1994) which is hereby incorporated by reference. Those skilled in the art are aware of other *hybrid* genes and the above example is not meant to limit the invention in any manner.

DETD(21) In . . . ingredient selected from B. thuringiensis var. kurstaki. A further embodiment comprises an active toxin selected from the group consisting of *CryIC*, *CrylA*(a), *CrylA*(b), Cry IIA, CrylA(c) and fragments and mixtures thereof including *hybrid* proteins.

CLMS(6) 6. . . . according to claim 2 wherein the active ingredient is a Bacillus thuringiensis crystal protein selected from the group consisting of *CryIC*, *CrylA*(a), *CrylA*(b), CrylA(c), CrylIA and CrylE proteins and mixtures or parts thereof.

US PAT NO: 5,851,545 [IMAGE AVAILABLE] L18: 2 of 4
DETD(5) The . . . be used in the invention. However, while not meant to limit the invention in any manner, preferred toxin proteins include *CrylA*, CrylB, *CryIC*, CryID, CryIE, CryIF, CryIG, CryII, CryIII, CrylV, CryIH, CryV, CtyA, CytB and any variants, mixtures or parts thereof. Particularly preferred toxins include *CryIC*, *CryIA*(a), *CrylA*(b), CrylA(c), CryIE, CryIIA and variants, mixtures and parts thereof.

DETD(6) Recombinant . . . proteins a particular Bacillus strain produces and the use of protein design to create a gene expressing a fusion or *hybrid* protein. An example of a *hybrid* gene is G27, containing fragments of different Cry proteins and specifically CrylE and CrylC. This protein is further described in. . . et al., Biotechnology 12:915-918 (1994) which is hereby incorporated by reference. Those skilled in the art are aware of other *hybrid* genes and the above example is not meant to limit the invention in any manner.

DETD(21) In . . . ingredient selected from B. thuringiensis var. kurstaki. A further embodiment comprises an active toxin selected from the group consisting of *CryIC*, *CrylA*(a), *CrylA*(b), Cry IIA, CrylA(c) and fragments and mixtures thereof including *hybrid* proteins.

US PAT NO: 5,804,393 [IMAGE AVAILABLE] L18: 3 of 4
BSUM(2) During . . . The different ICPs can be classified according to the scheme of Hofte and Whitely (1989). Known ICPs include CrylA(a), CrylA(b), *CrylA*(c) and *CrylC* toxins. The native crystal proteins are inactive protoxins which, after ingestion by the larvae, are dissolved in the alkaline insect. . .

BSUM(6) The fusion of the antibody producing cells with, typically, myeloma cells results in the formation of so-called *hybridoma* cells, with the aid of which monoclonal antibodies can be produced. The methods employed are described in the literature and. . .

BSUM(9) The . . . thuringiensis .delta.-endotoxins and their derivatives, or (c2) fusing spleen cells of the immunised animal with corresponding myeloma cells, selecting specific *hybridoma* cells and producing the desired antibodies using said *hybridoma* cells.

BSUM(16) An . . . the serum of the immunised animals, or spleen cells of the immunised animals are fused with corresponding myeloma cells, specific *hybridoma* cells are selected and the desired anti-idiotype antibody is produced using said *hybridoma* cells.

BSUM(19) An . . . thuringiensis .delta.-endotoxins and their derivatives, or (d2) fusing spleen cells of the immunised animal with corresponding myeloma cells, selecting specific *hybridoma* cells and producing the desired anti-idiotype antibodies using said *hybridoma* cells.

DETD(8) Example 1: CrylA(a), CryLA(b), *CrylA*(c) and *CrylC* toxins and their binding to BBM proteins from Heliothis and Spodoptera

DETD(9) After . . . 30 min at room temperature in TBSTM. The membrane is incubated overnight in 1.5 .mu.g/ml each of activated CrylA(a), CrylA(b), *CrylA*(c) and *CrylC* toxins and the unbound toxin is rem washing in TBST. Bound toxin is identified with the monoclonal antibody 82.1. . .

DETD(10) The three *CrylA* toxins and the *CrylC* toxin recognise one or more binding proteins in the of each insect species (Table 1).

US PAT NO: 5,702,703 [IMAGE AVAILABLE] L18: 4 of 4
BSUM(8) The . . . Pat. No. 4,448,885 and U.S. Pat. No. 4,467,036 both disclose the expression of a B.t. crystal protein in E. coli. *Hybrid* B.t. crystal protein genes have been constructed that exhibit increased toxicity and display an expanded host range to a target. . .

DETD(62) MVP.RTM. . . . as the active ingredient. DIPEL contains CrylA(a), CrylA(b), CrylA(c), and CrylIA Bacillus thuringiensis toxins as the active ingredients. XENTARI contains *CrylA*(a), *CrylA*(b), *CryIC*, and CryID Bacillus thuringiensis toxins as the active ingredients. AGREE contains *CrylA*(a), *CrylA*(c), *CryIC*, and CryID Bacillus thuringiensis toxins as the active ingredients.

(FILE 'HOME' ENTERED AT 09:07:01 ON 13 MAY 1997)

FILE 'CAPLUS' ENTERED AT 09:07:06 ON 13 MAY 1997
.1 3540 S THURINGIENSIS
.2 260631 S HYBRID OR FUS? OR CHIMER?
.3 209 S L1 AND L2
.4 56180 S TOXIN OR ENDOTOXIN OR CRYSTAL PROTEIN
.5 155 S L3 AND L4
.6 1240 S L2(5A)L4
.7 72 S L5 AND L6

.3 ANSWER 1 OF 209 CAPLUS COPYRIGHT 1997 ACS
.1 Novel lipolytic enzyme muteins designed for one-wash detergent compositions for the removal of fatty materials

.3 ANSWER 2 OF 209 CAPLUS COPYRIGHT 1997 ACS
.1 Recombinant lipases with C- and/or N-terminal extensions and their use in detergents

.3 ANSWER 3 OF 209 CAPLUS COPYRIGHT 1997 ACS
.1 Expression of cryIA(c) gene of Bacillus ***thuringiensis*** in transgenic chickpea plants inhibits development of pod-borer (Heliothis armigera) larvae

.3 ANSWER 4 OF 209 CAPLUS COPYRIGHT 1997 ACS
.1 Expression plasmids containing a root cortex-specific gene RD2 promoter from tobacco

.3 ANSWER 5 OF 209 CAPLUS COPYRIGHT 1997 ACS
.1 Recombinant lipases with C- and/or N-terminal extensions and their use in detergents

.3 ANSWER 6 OF 209 CAPLUS COPYRIGHT 1997 ACS
.1 Identification of a linkage group with a major effect on resistance to Bacillus ***thuringiensis*** Cry1Ac endotoxin in the tobacco budworm (Lepidoptera: Noctuidae)

.3 ANSWER 7 OF 209 CAPLUS COPYRIGHT 1997 ACS
.1 Cloning of a cryIIIA endotoxin gene of Bacillus ***thuringiensis*** var. tenebrionis and its transient expression in indica rice

.3 ANSWER 8 OF 209 CAPLUS COPYRIGHT 1997 ACS
.1 Expression of a bacterial luciferase marker gene in Bacillus species

.3 ANSWER 9 OF 209 CAPLUS COPYRIGHT 1997 ACS
.1 Triggered pore-forming agents

.3 ANSWER 10 OF 209 CAPLUS COPYRIGHT 1997 ACS
.1 Genetic analysis of cryIIIA gene expression in Bacillus ***thuringiensis***

.3 ANSWER 11 OF 209 CAPLUS COPYRIGHT 1997 ACS
.1 High-level transcription of the cryIIIA toxin gene of Bacillus ***thuringiensis*** depends on a second promoter located 600 bp upstream of the translational start site

.3 ANSWER 12 OF 209 CAPLUS COPYRIGHT 1997 ACS
.1 Cross-resistance of the diamondback moth indicates altered interactions with domain II of Bacillus ***thuringiensis*** toxins

.3 ANSWER 13 OF 209 CAPLUS COPYRIGHT 1997 ACS
.1 Different domains of Bacillus ***thuringiensis*** .delta.-endotoxins can bind to insect midgut membrane proteins on ligand blots

.3 ANSWER 14 OF 209 CAPLUS COPYRIGHT 1997 ACS
.1 A novel enzyme with .beta.-1,3-glucanase activity from Oerskovia xanthineolytica LLG109

.3 ANSWER 15 OF 209 CAPLUS COPYRIGHT 1997 ACS
.1 Novel strains of Bacillus that produce insecticidal proteins during vegetative growth and their genetic engineering

.3 ANSWER 16 OF 209 CAPLUS COPYRIGHT 1997 ACS
.1 Recombinant cyanobacteria producing CryIVD endotoxin and its use as biopesticide against Diptera

.3 ANSWER 17 OF 209 CAPLUS COPYRIGHT 1997 ACS
.1 STAB-SD: a Shine-Dalgarno sequence in the 5' untranslated region is a determinant of mRNA stability

.3 ANSWER 18 OF 209 CAPLUS COPYRIGHT 1997 ACS
.1 Identification of a Bacillus ***thuringiensis*** gene that positively regulates transcription of the phosphatidylinositol-specific phospholipase C gene at the onset of the stationary phase

.3 ANSWER 19 OF 209 CAPLUS COPYRIGHT 1997 ACS
.1 Lepidopteran pesticidal compositions comprising ***chimeric*** CryIF and CryIA(c) .delta.-endotoxins

.3 ANSWER 20 OF 209 CAPLUS COPYRIGHT 1997 ACS
.1 Domain III substitution in Bacillus ***thuringiensis*** delta-endotoxin CryIA(b) results in superior toxicity for Spodoptera exigua and altered membrane protein recognition

.3 ANSWER 21 OF 209 CAPLUS COPYRIGHT 1997 ACS
.1 Analysis of cryIAa expression in sigE and sigK mutants of Bacillus ***thuringiensis***

.3 ANSWER 22 OF 209 CAPLUS COPYRIGHT 1997 ACS
.1 Antibodies which bind to insect gut proteins and their use in preparation of immunotoxins

.3 ANSWER 23 OF 209 CAPLUS COPYRIGHT 1997 ACS
.1 Transfer and transcriptional expression of coleopteran cryIIIB endotoxin gene of Bacillus ***thuringiensis*** in eggplant

.3 ANSWER 24 OF 209 CAPLUS COPYRIGHT 1997 ACS
.1 Comparative study in three systems of heterologous expression of recombinant .delta.-endotoxins from Bacillus ***thuringiensis*** in Escherichia coli

.3 ANSWER 25 OF 209 CAPLUS COPYRIGHT 1997 ACS
.1 Induced synthesis of a Coleoptera-specific insecticidal protein of Bacillus ***thuringiensis*** in Pseudomonoas putida cells

.3 ANSWER 26 OF 209 CAPLUS COPYRIGHT 1997 ACS
.1 Biologically safe plant transformation system using transposable element and transposase gene

.3 ANSWER 27 OF 209 CAPLUS COPYRIGHT 1997 ACS
.1 Microbial populations, fungal species diversity and plant pathogenlevels in field plots of potato plants expressing the Bacillus ***thuringiensis*** var. tenebrionis endotoxin

.3 ANSWER 28 OF 209 CAPLUS COPYRIGHT 1997 ACS
.1 Recombinant preparation of ***chimeric*** Bacillus ***thuringiensis*** .delta.-endotoxin of cryIC and cryIA(b) with improved toxicity

.3 ANSWER 29 OF 209 CAPLUS COPYRIGHT 1997 ACS
.1 ***Chimeric*** Bacillus ***thuringiensis*** .delta.-endotoxin expression in Pseudomonas fluorescens and its improvement

3 ANSWER 30 OF 209 CAPLUS COPYRIGHT 1997 ACS
I Construction of expression plasmids containing a root-specific gene promoter from tobacco

3 ANSWER 31 OF 209 CAPLUS COPYRIGHT 1997 ACS
I Development of insect resistance in tomato plants expressing the.delta.-endotoxin gene of Bacillus ***thuringiensis*** subsp. tenebrionis

3 ANSWER 32 OF 209 CAPLUS COPYRIGHT 1997 ACS
I Domain III exchanges of Bacillus ***thuringiensis*** cryIa toxins affect binding to different gypsy moth midgut receptors

3 ANSWER 33 OF 209 CAPLUS COPYRIGHT 1997 ACS
I ***Hybrid*** toxins of Bacillus ***thuringiensis***

3 ANSWER 34 OF 209 CAPLUS COPYRIGHT 1997 ACS
I Insecticidal proteins constructed from Bacillus ***thuringiensis*** .delta.-endotoxin and Androctonus australis neurotoxin AaHIT

3 ANSWER 35 OF 209 CAPLUS COPYRIGHT 1997 ACS
I Cloning of capsular operon of anthrax microbe and its use for identification of virulent strains of Bacillus anthracis

3 ANSWER 36 OF 209 CAPLUS COPYRIGHT 1997 ACS
I Transfer of an insecticidal protein gene of Bacillus ***thuringiensis*** into plant-colonizing Azospirillum

3 ANSWER 37 OF 209 CAPLUS COPYRIGHT 1997 ACS
I Development of Bacillus ***thuringiensis*** CryIC resistance by Spodoptera exigua (Huebner) (Lepidoptera: Noctuidae)

3 ANSWER 38 OF 209 CAPLUS COPYRIGHT 1997 ACS
I Transcriptional regulation of the cryIVD gene operon from Bacillus ***thuringiensis*** subsp. israelensis

3 ANSWER 39 OF 209 CAPLUS COPYRIGHT 1997 ACS
I Membrane permeabilization by Listeria monocytogenes phosphatidylinositol-specific phospholipase C is independent of phospholipid hydrolysis and cooperative with listeriolysin O

3 ANSWER 40 OF 209 CAPLUS COPYRIGHT 1997 ACS
I Amplification of a ***chimeric*** Bacillus gene in chloroplasts leads to an extraordinary level of an insecticidal protein in tobacco

3 ANSWER 41 OF 209 CAPLUS COPYRIGHT 1997 ACS
I Transgenic tobacco plants with efficient insect resistance

3 ANSWER 42 OF 209 CAPLUS COPYRIGHT 1997 ACS
I The effect of toxin-producing Rhizobium strains, on larvae of Sitona flavescens feeding on legume roots and nodules. [Erratum to document cited in CA121:274435]

3 ANSWER 43 OF 209 CAPLUS COPYRIGHT 1997 ACS
I Expression of Bacillus ***thuringiensis*** .delta.-endotoxin gene with recombinant baculovirus in insect cell

L3 ANSWER 44 OF 209 CAPLUS COPYRIGHT 1997 ACS
I Cell-targeted lytic pore-forming agents for destroying unwanted cells associated with pathological conditions, such as metastatic cancer

L3 ANSWER 45 OF 209 CAPLUS COPYRIGHT 1997 ACS
I Specificity domain localization of Bacillus ***thuringiensis*** insecticidal toxins is highly dependent on the bioassay system

L3 ANSWER 46 OF 209 CAPLUS COPYRIGHT 1997 ACS
I Insecticidal ***fusion*** proteins of Bacillus ***thuringiensis*** var. kurstaki HD-1

L3 ANSWER 47 OF 209 CAPLUS COPYRIGHT 1997 ACS
I Insect resistance of transgenic plants that express modified Bacillus ***thuringiensis*** cryIA(b) and cryIC genes: a resistance management strategy

L3 ANSWER 48 OF 209 CAPLUS COPYRIGHT 1997 ACS
I Protoplast ***fusion*** of Bacillus subtilis and Bacillus ***thuringiensis*** for breeding of pesticidal strains against plant pathogens

L3 ANSWER 49 OF 209 CAPLUS COPYRIGHT 1997 ACS
I The effect of toxin-producing Rhizobium strains, on larvae of Sitona flavescens feeding on legume roots and nodules

L3 ANSWER 50 OF 209 CAPLUS COPYRIGHT 1997 ACS
I Expression of the insecticidal crystal protein gene from a Gram-positive Bacillus ***thuringiensis*** in a Gram-negative Pseudomonas fluorescens mediated by protoplast ***fusion***

L3 ANSWER 51 OF 209 CAPLUS COPYRIGHT 1997 ACS
I Recombinant Bacillus ***thuringiensis*** crystal proteins with new properties: possibilities for resistance management

L3 ANSWER 52 OF 209 CAPLUS COPYRIGHT 1997 ACS
I Location of a lepidopteran specificity region in insecticidal crystal protein CryIIA from Bacillus ***thuringiensis***

L3 ANSWER 53 OF 209 CAPLUS COPYRIGHT 1997 ACS
I Biochemical and morphological changes in rat muscle cultures caused by 28,000 mol. wt toxin of Bacillus ***thuringiensis*** israelensis

L3 ANSWER 54 OF 209 CAPLUS COPYRIGHT 1997 ACS
I Cloning of a new cryIA(a) gene from Bacillus ***thuringiensis*** strain FU-2-7 and analysis of ***chimeric*** CryIA(a) proteins for toxicity

L3 ANSWER 55 OF 209 CAPLUS COPYRIGHT 1997 ACS
I Cyclohexane carboxylic acid phenyl ester hydrolase and its preparation by fermentation

L3 ANSWER 56 OF 209 CAPLUS COPYRIGHT 1997 ACS
I Structural and functional analysis of the promoter region involved in full expression of the cryIIIA toxin gene of Bacillus ***thuringiensis***

L3 ANSWER 57 OF 209 CAPLUS COPYRIGHT 1997 ACS
I Expression in Bacillus subtilis of the Bacillus ***thuringiensis*** cryIIIA toxin gene is not dependent on a sporulation-specific sigma factor and is increased in a spo0A mutant

L3 ANSWER 58 OF 209 CAPLUS COPYRIGHT 1997 ACS
I Insect tolerance of transgenic Populus nigra plants transformed with Bacillus ***thuringiensis*** toxin gene

L3 ANSWER 59 OF 209 CAPLUS COPYRIGHT 1997 ACS
I Methods for the production of ***hybrid*** seeds

L3 ANSWER 60 OF 209 CAPLUS COPYRIGHT 1997 ACS
I Intracellular proteolysis and limited diversity of the Bacillus ***thuringiensis*** CryIA family of the insecticidal crystal proteins

L3 ANSWER 61 OF 209 CAPLUS COPYRIGHT 1997 ACS
I Use of an operon ***fusion*** to induce expression and crystallization of a Bacillus ***thuringiensis*** .delta.-endotoxin encoded by a cryptic gene

L3 ANSWER 62 OF 209 CAPLUS COPYRIGHT 1997 ACS
I Low levels of expression of wild type Bacillus ***thuringiensis*** var. kurstaki cryIA (c) sequences in transgenic walnut somatic embryos

L3 ANSWER 63 OF 209 CAPLUS COPYRIGHT 1997 ACS
I Agrobacterium-mediated transformation of ***hybrid*** poplar suspension cultures and regeneration of transformed plants

3 ANSWER 64 OF 209 CAPLUS COPYRIGHT 1997 ACS
I Microgranulated products usable in combination with bacterial inoculums, in agriculture.

3 ANSWER 65 OF 209 CAPLUS COPYRIGHT 1997 ACS
I IS231V and W from Bacillus ***thuringiensis*** subsp. israelensis, two distant members of the IS231 family of insertion sequences

3 ANSWER 66 OF 209 CAPLUS COPYRIGHT 1997 ACS
I Primary structure of cryX, the novel .delta.-endotoxin-related gene from Bacillus ***thuringiensis*** spp. galleriae

3 ANSWER 67 OF 209 CAPLUS COPYRIGHT 1997 ACS
I Expression of cryIVA and cryIVB genes, independently or in combination, in a crystal-negative strain of Bacillus ***thuringiensis*** subsp. israelensis

3 ANSWER 68 OF 209 CAPLUS COPYRIGHT 1997 ACS
I Performance of Pirate, insecticide-miticide, against cotton pests, in the mid-south in 1992

3 ANSWER 69 OF 209 CAPLUS COPYRIGHT 1997 ACS
I Binding of an engineered 130-kDa insecticidal protein of Bacillus ***thuringiensis*** var. israelensis to insect cell lines

3 ANSWER 70 OF 209 CAPLUS COPYRIGHT 1997 ACS
I Use of maize hsp70 intron to enhance ***chimeric*** gene expression in monocots

3 ANSWER 71 OF 209 CAPLUS COPYRIGHT 1997 ACS
I Transgenic cabbage plants with insect tolerance

3 ANSWER 72 OF 209 CAPLUS COPYRIGHT 1997 ACS
I Construction of a gene for a ***hybrid*** protein based on Bacillus ***thuringiensis*** .delta.-endotoxin CryIA(a) and CryIIIA sequences and expression of its derivatives in Escherichia coli

3 ANSWER 73 OF 209 CAPLUS COPYRIGHT 1997 ACS
I Genetic transformation of potato with Bacillus ***thuringiensis*** HD 73 CryIA(c) gene and development of insect resistant plants

3 ANSWER 74 OF 209 CAPLUS COPYRIGHT 1997 ACS
I A novel .alpha.-amylase gene promoter of Bacillus, its cloning and use for protein recombinant manufacture

3 ANSWER 75 OF 209 CAPLUS COPYRIGHT 1997 ACS
I The reconstruction and expression of a Bacillus ***thuringiensis*** cryIIIA gene in protoplasts and potato plants

3 ANSWER 76 OF 209 CAPLUS COPYRIGHT 1997 ACS
I Full expression of the cryIIIA toxin gene of Bacillus ***thuringiensis*** requires a distant upstream DNA sequence affecting transcription

L3 ANSWER 77 OF 209 CAPLUS COPYRIGHT 1997 ACS
TI Expression of endotoxin gene from Bacillus ***thuringiensis*** with insect baculovirus transfer vector in Escherichia coli

L3 ANSWER 78 OF 209 CAPLUS COPYRIGHT 1997 ACS
TI Transformation of Liquidambar styraciflua using Agrobacterium tumefaciens

L3 ANSWER 79 OF 209 CAPLUS COPYRIGHT 1997 ACS
TI Effects of Bacillus ***thuringiensis*** var. israelensis 20-kDa protein on production of the Bti 130-kDa crystal protein in Escherichia coli

L3 ANSWER 80 OF 209 CAPLUS COPYRIGHT 1997 ACS
TI Simple method to evaluate sterilization of recombinant Pseudomonas carrying insecticidal protein gene

L3 ANSWER 81 OF 209 CAPLUS COPYRIGHT 1997 ACS
TI Engineering for apple and walnut resistance to codling moth

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TI Synthetic genes for delta-endotoxins optimized for expression in maize

L3 ANSWER 83 OF 209 CAPLUS COPYRIGHT 1997 ACS
TI Field performance of elite transgenic maize plants expressing an insecticidal protein derived from Bacillus ***thuringiensis***

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TI Stable transformation of Picea glauca by particle acceleration

L3 ANSWER 85 OF 209 CAPLUS COPYRIGHT 1997 ACS
TI Expression of mutated .delta.-endotoxin gene of Bacillus ***thuringiensis*** subsp. tenebrionis in E. coli and insecticidal activity against Coleopteran insects

L3 ANSWER 86 OF 209 CAPLUS COPYRIGHT 1997 ACS
TI Transfer of Bacillus ***thuringiensis*** toxin gene into Bacillus subtilis and its inoculation effects

L3 ANSWER 87 OF 209 CAPLUS COPYRIGHT 1997 ACS
TI Gene expression cassette containing somatotropin gene exon 5 non-coding sequence for expression of cDNA in animal cells

L3 ANSWER 88 OF 209 CAPLUS COPYRIGHT 1997 ACS
TI Expression of a ***hybrid*** gene for bifunctional insect toxin-glucuronidase protein in transgenic tobacco

L3 ANSWER 89 OF 209 CAPLUS COPYRIGHT 1997 ACS
TI Insecticidal protein cryIA(b) manufacture with Bacillus for control of Lepidoptera

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L3 ANSWER 90 OF 209 CAPLUS COPYRIGHT 1997 ACS
TI Expression of a ***chimeric*** CaMV 35S Bacillus ***thuringiensis*** insecticidal protein gene in transgenic tobacco. [Erratum to document cited in CA118(3):17151c]

L3 ANSWER 91 OF 209 CAPLUS COPYRIGHT 1997 ACS
TI A sporulation-dependent promoter of exoproteinase of Bacillus ***thuringiensis***

L3 ANSWER 92 OF 209 CAPLUS COPYRIGHT 1997 ACS
TI Suppression of protein structure destabilizing mutations in Bacillus ***thuringiensis*** .delta.-endotoxins by second site mutations

L3 ANSWER 93 OF 209 CAPLUS COPYRIGHT 1997 ACS
TI Transgenic tomato plants expressing insecticidal activity against coleopteran larvae

L3 ANSWER 94 OF 209 CAPLUS COPYRIGHT 1997 ACS
TI Expression of a ***chimeric*** CaMV 35S Bacillus ***thuringiensis*** insecticidal protein gene in transgenic tobacco

L3 ANSWER 95 OF 209 CAPLUS COPYRIGHT 1997 ACS
TI Strong ***hybrid*** promoters for heterologous gene expression in Bacillus

L3 ANSWER 96 OF 209 CAPLUS COPYRIGHT 1997 ACS
TI Transgenic rice plant of a superior Chinese cultivar Zhonghua No. 11 containing the B. t. .delta.-endotoxin gene in its genome

L3 ANSWER 97 OF 209 CAPLUS COPYRIGHT 1997 ACS
TI Isolation and partial characterization of binding proteins for immobilized delta endotoxin from solubilized brush border membrane vesicles of the silkworm, Bombyx mori, and the common cutworm, Spodoptera litura

L3 ANSWER 98 OF 209 CAPLUS COPYRIGHT 1997 ACS
TI Arabidopsis thaliana small subunit leader and transit peptide enhance the expression of Bacillus ***thuringiensis*** proteins in transgenic plants

L3 ANSWER 99 OF 209 CAPLUS COPYRIGHT 1997 ACS
TI Conventional and alternative insecticides, including a granular formulation of Bacillus ***thuringiensis*** var. kurstaki, for the control of Busseola ***fusca*** (Fuller) (Lepidoptera: Noctuidae) in Kenya

L3 ANSWER 100 OF 209 CAPLUS COPYRIGHT 1997 ACS
TI Evaluation of aerial applications of acephate and other insecticides for control of cone and seed insects in southern pine seed orchards

L3 ANSWER 101 OF 209 CAPLUS COPYRIGHT 1997 ACS
TI Extending the host range of insecticidal proteins using peptides that bind gut cells

L3 ANSWER 102 OF 209 CAPLUS COPYRIGHT 1997 ACS
TI Overproduction, purification and characterization of M.cntdot.HinfI methyltransferase and its deletion mutant

L3 ANSWER 103 OF 209 CAPLUS COPYRIGHT 1997 ACS
TI Cloning and expression of the cryIVD gene of Bacillus ***thuringiensis*** subsp. israelensis in the cyanobacterium Agmenellum quadruplicatum PR-6 and its resulting larvicidal activity

L3 ANSWER 104 OF 209 CAPLUS COPYRIGHT 1997 ACS
TI Strains of Bacillus ***thuringiensis*** and their genes encoding insecticidal toxins

L3 ANSWER 105 OF 209 CAPLUS COPYRIGHT 1997 ACS
TI *Translation enhancing properties of the 5'-leader of potato virus X genomic RNA*

L3 ANSWER 106 OF 209 CAPLUS COPYRIGHT 1997 ACS
TI The C-terminal domain of the toxic fragment of a Bacillus ***thuringiensis*** crystal protein determines receptor binding

L3 ANSWER 107 OF 209 CAPLUS COPYRIGHT 1997 ACS
TI Construction of genes for bifunctional derivatives of Bacillus ***thuringiensis*** var. kurstaki insect toxin for expression in transgenic plants

L3 ANSWER 108 OF 209 CAPLUS COPYRIGHT 1997 ACS
TI Production of insect resistant potato by genetic transformation with a .delta.-endotoxin gene from Bacillus ***thuringiensis*** var. kurstaki

L3 ANSWER 109 OF 209 CAPLUS COPYRIGHT 1997 ACS
TI Isolation and cloning of Bacillus ***thuringiensis*** var Kurstaki HD73 toxin gene and construction of a ***chimeric*** gene for expression in plants.

L3 ANSWER 110 OF 209 CAPLUS COPYRIGHT 1997 ACS
TI Cloning and expression of Lactococcus MSP protein gene, and Escherichia-lactococcus shuttle vectors

L3 ANSWER 111 OF 209 CAPLUS COPYRIGHT 1997 ACS
TI A temperature-stable Bacillus ***thuringiensis*** .delta.-endotoxin analog

L3 ANSWER 112 OF 209 CAPLUS COPYRIGHT 1997 ACS
TI Development of insect resistant plants

L3 ANSWER 113 OF 209 CAPLUS COPYRIGHT 1997 ACS
TI Agricultural chemical-producing endosymbiotic microorganisms produced by protoplast ***fusion***

L3 ANSWER 114 OF 209 CAPLUS COPYRIGHT 1997 ACS
TI *Transgenic plants expressing insecticidal proteins*

L3 ANSWER 115 OF 209 CAPLUS COPYRIGHT 1997 ACS
TI Generation of functional Bacillus ***thuringiensis*** toxin ***hybrid*** genes by in vivo recombination

L3 ANSWER 116 OF 209 CAPLUS COPYRIGHT 1997 ACS
TI In vivo generation of hybrids between two Bacillus ***thuringiensis*** insect-toxin-encoding genes

L3 ANSWER 117 OF 209 CAPLUS COPYRIGHT 1997 ACS
TI Functional domains of Bacillus ***thuringiensis*** insecticidal crystal proteins. Refinement of Heliothis virescens and Trichoplusia ni specificity domains on CryIA(c)

L3 ANSWER 118 OF 209 CAPLUS COPYRIGHT 1997 ACS
TI Insectidical activity of Bacillus ***thuringiensis*** ***chimeric*** protoxins

L3 ANSWER 119 OF 209 CAPLUS COPYRIGHT 1997 ACS
TI High-level expression in Escherichia coli and rapid purification of phosphatidylinositol-specific phospholipase C from Bacillus cereus and Bacillus ***thuringiensis***

L3 ANSWER 120 OF 209 CAPLUS COPYRIGHT 1997 ACS
TI Activation of a cryptic crystal protein gene of Bacillus ***thuringiensis*** subspecies kurstaki by gene ***fusion*** and determination of the crystal protein insecticidal specificity

L3 ANSWER 121 OF 209 CAPLUS COPYRIGHT 1997 ACS
TI New functional Bacillus ***thuringiensis*** .delta.-endotoxin ***hybrid*** genes obtained by in vivo recombination

L3 ANSWER 122 OF 209 CAPLUS COPYRIGHT 1997 ACS
TI Transgenic plants for the prevention of development of insects resistant to Bacillus ***thuringiensis*** toxins

L3 ANSWER 123 OF 209 CAPLUS COPYRIGHT 1997 ACS
TI *Stable transformation of Populus and incorporation of pest resistance by electric discharge particle acceleration*

L3 ANSWER 124 OF 209 CAPLUS COPYRIGHT 1997 ACS
TI Cloning and expression of gas vesicle protein genes of Pseudoanabaena in Bacillus thueinglensis israelensis

L3 ANSWER 125 OF 209 CAPLUS COPYRIGHT 1997 ACS
TI Expression of Bacillus ***thuringiensis*** delta-endotoxin in transgenic plants of Nicotiana tabacum

L3 ANSWER 126 OF 209 CAPLUS COPYRIGHT 1997 ACS
TI Bacillus ***thuringiensis*** strains producing novel endotoxins, the endotoxin genes, and transgenic plants containing the gene

L3 ANSWER 127 OF 209 CAPLUS COPYRIGHT 1997 ACS
TI *Novel .delta.-endotoxin gene of Bacillus ***thuringiensis*** kurstaki and expression of ***chimeric*** .delta.-endotoxin genes containing it*

L3 ANSWER 128 OF 209 CAPLUS COPYRIGHT 1997 ACS
TI Production of soluble recombinant ricin

L3 ANSWER 129 OF 209 CAPLUS COPYRIGHT 1997 ACS
TI Larvicidal activity of ***chimeric*** Bacillus ***thuringiensis*** protoxins

L3 ANSWER 130 OF 209 CAPLUS COPYRIGHT 1997 ACS
TI Specificity-determining regions of a lepidopteran-specific insecticidal protein produced by Bacillus ***thuringiensis***

L3 ANSWER 131 OF 209 CAPLUS COPYRIGHT 1997 ACS
TI Cloning of Bacillus ***thuringiensis*** bt4 and bt18 genes, and lepidoptera-resistant plants containing these genes

L3 ANSWER 132 OF 209 CAPLUS COPYRIGHT 1997 ACS
TI ***Hybrid*** pesticidal protein toxins, microorganisms producing them, and use of the toxins to control insects

L3 ANSWER 133 OF 209 CAPLUS COPYRIGHT 1997 ACS

TI Differential expression of the 3 .delta.-endotoxin genes in Bacillus ***thuringiensis*** subsp. kurstaki HD1

L3 ANSWER 134 OF 209 CAPLUS COPYRIGHT 1997 ACS
TI Location of the dipteran specificity region in a lepidopteran-dipteran crystal protein from Bacillus ***thuringiensis***

L3 ANSWER 135 OF 209 CAPLUS COPYRIGHT 1997 ACS
TI Heterologous expression of a mutated toxin gene from Bacillus ***thuringiensis*** subsp. tenebrionis

L3 ANSWER 136 OF 209 CAPLUS COPYRIGHT 1997 ACS
TI ***Chimeric*** Bacillus ***thuringiensis*** .delta.-endotoxin gene

L3 ANSWER 137 OF 209 CAPLUS COPYRIGHT 1997 ACS
TI Expression of the Bacillus ***thuringiensis*** crystal protein gene in Pseudomonas isolated from rhizosphere soil of Korean crops

L3 ANSWER 138 OF 209 CAPLUS COPYRIGHT 1997 ACS
TI Novel ***hybrid*** Bacillus .delta.-endotoxin for control of Lepidopteran insects

L3 ANSWER 139 OF 209 CAPLUS COPYRIGHT 1997 ACS
TI Transgenic rice plants produced by direct uptake of .delta.-endotoxin protein gene from Bacillus ***thuringiensis*** into rice protoplasts

L3 ANSWER 140 OF 209 CAPLUS COPYRIGHT 1997 ACS
TI Construction of ***chimeric*** insecticidal proteins between the 130-kDa and 135-kDa proteins of Bacillus ***thuringiensis*** subsp. aizawai for analysis of structure-function relationship

L3 ANSWER 141 OF 209 CAPLUS COPYRIGHT 1997 ACS
TI A translation ***fusion*** product of two different insecticidal crystal protein genes of Bacillus ***thuringiensis*** exhibits an enlarged insecticidal spectrum

L3 ANSWER 142 OF 209 CAPLUS COPYRIGHT 1997 ACS
TI Potentiation of Bacillus ***thuringiensis*** insecticidal activity by serine protease inhibitors

L3 ANSWER 143 OF 209 CAPLUS COPYRIGHT 1997 ACS
TI Application of techniques of genetic exchange and genetic engineering to the improvement of the insecticidal properties of Bacillus ***thuringiensis***

L3 ANSWER 144 OF 209 CAPLUS COPYRIGHT 1997 ACS
TI Cloning and expression in microorganisms of endotoxin gene of Bacillus ***thuringiensis*** tenebrionis

L3 ANSWER 145 OF 209 CAPLUS COPYRIGHT 1997 ACS
TI Intergeneric protoplast ***fusion*** between Agrobacterium tumefaciens and Bacillus ***thuringiensis*** subsp. kurstaki

L3 ANSWER 146 OF 209 CAPLUS COPYRIGHT 1997 ACS
TI ***Chimeric*** .delta.-endotoxins of Bacillus ***thuringiensis*** with novel host ranges and their manufacture in Escherichia coli

L3 ANSWER 147 OF 209 CAPLUS COPYRIGHT 1997 ACS
TI Plants transformed with a gene for an insecticidal protein from Bacillus ***thuringiensis***

L3 ANSWER 148 OF 209 CAPLUS COPYRIGHT 1997 ACS
TI Insecticidal activity of a peptide containing the 30th to 695th amino acid residues of the 130-kDa protein of Bacillus ***thuringiensis*** var. israelensis

L3 ANSWER 149 OF 209 CAPLUS COPYRIGHT 1997 ACS
TI Cloning and expression of genes encoding proteins with larvicidal activity against Lepidoptera

L3 ANSWER 150 OF 209 CAPLUS COPYRIGHT 1997 ACS
TI Accumulation of the insecticidal crystal protein of Bacillus ***thuringiensis*** subsp. kurstaki in post-exponential-phase Bacillus subtilis

L3 ANSWER 151 OF 209 CAPLUS COPYRIGHT 1997 ACS
TI Novel .delta.-endotoxin gene from Bacillus ***thuringiensis*** israelensis and its expression and use as insecticide

L3 ANSWER 152 OF 209 CAPLUS COPYRIGHT 1997 ACS
TI A 20-kilodalton protein is required for efficient production of the Bacillus ***thuringiensis*** subsp. israelensis 27-kilodalton crystal protein in Escherichia coli

L3 ANSWER 153 OF 209 CAPLUS COPYRIGHT 1997 ACS
TI Monoclonal antibodies against the 65-kilodalton mosquitocidal protein of the Bacillus ***thuringiensis*** strain PG-14 (serotype 8a:8b)

L3 ANSWER 154 OF 209 CAPLUS COPYRIGHT 1997 ACS
TI Control of sewage filter flies using Bacillus ***thuringiensis*** var. israelensis - II. Full scale trials

L3 ANSWER 155 OF 209 CAPLUS COPYRIGHT 1997 ACS
TI Location of the Bombyx mori specificity domain on a Bacillus ***thuringiensis*** .delta.-endotoxin protein

L3 ANSWER 156 OF 209 CAPLUS COPYRIGHT 1997 ACS
TI Regeneration of Zea mays protoplasts containing a cloned Bacillus ***thuringiensis*** crystal protein gene

L3 ANSWER 157 OF 209 CAPLUS COPYRIGHT 1997 ACS
TI ***Chimeric*** pesticide proteins of Bacillus ***thuringiensis*** and their recombinant manufacture

L3 ANSWER 158 OF 209 CAPLUS COPYRIGHT 1997 ACS
TI Thaumatin II: a simple marker gene for use in plants

L3 ANSWER 159 OF 209 CAPLUS COPYRIGHT 1997 ACS
TI Plasmids for heterologous protein production and secretion in Streptomycetes

L3 ANSWER 160 OF 209 CAPLUS COPYRIGHT 1997 ACS
TI Novel Bacillus ***thuringiensis*** with altered insecticidal activities prepared by protoplast ***fusion***

L3 ANSWER 161 OF 209 CAPLUS COPYRIGHT 1997 ACS
TI Expression of Bacillus endotoxin gene in cyanobacteria, and use of the transformants as an insecticide

L3 ANSWER 162 OF 209 CAPLUS COPYRIGHT 1997 ACS
TI Monoclonal antibodies to crystal protein of Bacillus ***thuringiensis*** israelensis

L3 ANSWER 163 OF 209 CAPLUS COPYRIGHT 1997 ACS
TI Obtaining a ***hybrid*** for a new insecticide by means of protoplast ***fusion***

L3 ANSWER 164 OF 209 CAPLUS COPYRIGHT 1997 ACS
TI Engineering of insect resistant plants using a B. ***thuringiensis*** gene

L3 ANSWER 165 OF 209 CAPLUS COPYRIGHT 1997 ACS
TI Introduction of the Streptococcus faecalis transposon Tn916 into Bacillus ***thuringiensis*** subsp. israelensis

L3 ANSWER 166 OF 209 CAPLUS COPYRIGHT 1997 ACS
TI Application of genetic engineering technology in the creation of tobaccos resistant to insects

L3 ANSWER 167 OF 209 CAPLUS COPYRIGHT 1997 ACS
TI Enhancement of the expression of genes in bacteria by transformation with a vector containing an enhancing DNA sequence

L3 ANSWER 168 OF 209 CAPLUS COPYRIGHT 1997 ACS
TI Insect resistance in transgenic plants expressing Bacillus ***thuringiensis*** toxin genes

L3 ANSWER 169 OF 209 CAPLUS COPYRIGHT 1997 ACS
TI Identification of flavones which induce expression of Rhizobium or Bradyrhizobium legume-nodulating genes in legume extracts

L3 ANSWER 170 OF 209 CAPLUS COPYRIGHT 1997 ACS
TI Sequence of a lepidopteran toxin gene of Bacillus ***thuringiensis*** subsp kurstaki NRD-12

L3 ANSWER 171 OF 209 CAPLUS COPYRIGHT 1997 ACS
TI *Effect of fungicides on the germination, root/shoot growth and incidence of seed-borne pathogens in rice*

L3 ANSWER 172 OF 209 CAPLUS COPYRIGHT 1997 ACS
TI ***Fusion*** proteins with both insecticidal and neomycin phosphotransferase II activity

L3 ANSWER 173 OF 209 CAPLUS COPYRIGHT 1997 ACS
TI Cloning and expression of two homologous genes of Bacillus ***thuringiensis*** subsp. israelensis which encode 130-kilodalton mosquitocidal proteins

L3 ANSWER 174 OF 209 CAPLUS COPYRIGHT 1997 ACS
TI Insect tolerant transgenic tomato plants

L3 ANSWER 175 OF 209 CAPLUS COPYRIGHT 1997 ACS
TI Bacillus ***thuringiensis*** .delta.-endotoxin expressed in transgenic Nicotiana tabacum provides resistance to Lepidopteran insects

L3 ANSWER 176 OF 209 CAPLUS COPYRIGHT 1997 ACS
TI Homologous and heterologous transception of Cry+ plasmids in Bacillus ***thuringiensis***

L3 ANSWER 177 OF 209 CAPLUS COPYRIGHT 1997 ACS
TI Agricultural-chemical-producing endosymbiotic microorganisms and method for preparing and using them

L3 ANSWER 178 OF 209 CAPLUS COPYRIGHT 1997 ACS
TI Expression of a cloned Bacillus ***thuringiensis*** crystal protein gene in Escherichia coli

L3 ANSWER 179 OF 209 CAPLUS COPYRIGHT 1997 ACS
TI Insecticidal .delta.-endotoxin production by genetically engineered Escherichia coli

L3 ANSWER 180 OF 209 CAPLUS COPYRIGHT 1997 ACS
TI ***Hybrid*** Bacillus ***thuringiensis*** producing .delta.-endotoxins of kurstaki and tenebrionis strains

L3 ANSWER 181 OF 209 CAPLUS COPYRIGHT 1997 ACS
TI New strains of Bacillus ***thuringiensis*** produced by protoplast ***fusion***

L3 ANSWER 182 OF 209 CAPLUS COPYRIGHT 1997 ACS
TI Alkaline phosphatase-mediated processing and secretion of recombinant proteins, DNA sequences for use therein and cells transformed using such sequences

L3 ANSWER 183 OF 209 CAPLUS COPYRIGHT 1997 ACS
TI Modifying plants by genetic engineering to combat or control insects

L3 ANSWER 184 OF 209 CAPLUS COPYRIGHT 1997 ACS
TI *Development of an improved ELISA for antibody detection and use in production of a hybridoma secreting a monoclonal antibody specific for crystal protein of Bacillus* ***thuringiensis*** *ssp. israelensis*

L3 ANSWER 185 OF 209 CAPLUS COPYRIGHT 1997 ACS
TI *Cloning and expression of the lepidopteran toxin produced by Bacillus* ***thuringiensis*** *var.* ***thuringiensis*** *in Escherichia coli*

L3 ANSWER 186 OF 209 CAPLUS COPYRIGHT 1997 ACS
TI Identification of a positive retroregulator that stabilizes mRNAs in bacteria

L3 ANSWER 187 OF 209 CAPLUS COPYRIGHT 1997 ACS
TI Antimicrobial activity of mycotoxins

L3 ANSWER 188 OF 209 CAPLUS COPYRIGHT 1997 ACS
TI Compositions containing biosynthetic pesticidal products and their use

L3 ANSWER 189 OF 209 CAPLUS COPYRIGHT 1997 ACS
TI Delineation of a toxin-encoding segment of a Bacillus ***thuringiensis*** crystal protein gene

L3 ANSWER 190 OF 209 CAPLUS COPYRIGHT 1997 ACS
TI Molecular cloning of the delta-endotoxin gene of Bacillus ***thuringiensis*** var. israelensis

L3 ANSWER 191 OF 209 CAPLUS COPYRIGHT 1997 ACS
TI Recent aspects of genetic manipulation in Bacillus ***thuringiensis***

L3 ANSWER 192 OF 209 CAPLUS COPYRIGHT 1997 ACS
TI Bacillus ***thuringiensis*** crystal protein in Escherichia coli

L3 ANSWER 193 OF 209 CAPLUS COPYRIGHT 1997 ACS
TI Cloning and expression in Escherichia coli of the insecticidal .delta.-endotoxin gene of Bacillus ***thuringiensis*** var. israelensis

L3 ANSWER 194 OF 209 CAPLUS COPYRIGHT 1997 ACS
TI Protecting bacteria

L3 ANSWER 195 OF 209 CAPLUS COPYRIGHT 1997 ACS
TI Microbiological implications of electric field effects. Part VIII. ***Fusion*** of Bacillus ***thuringiensis*** protoplasts by high electric field pulses

L3 ANSWER 196 OF 209 CAPLUS COPYRIGHT 1997 ACS
TI Interspecific recombinants of Bacillus ***thuringiensis*** times. Bacillus cereus

L3 ANSWER 197 OF 209 CAPLUS COPYRIGHT 1997 ACS
TI *Mycotoxin sensitivity of Bacillus* ***thuringiensis***

L3 ANSWER 198 OF 209 CAPLUS COPYRIGHT 1997 ACS
TI Isolation of a DNA sequence related to several plasmids from Bacillus ***thuringiensis*** after a mating involving the Streptococcus faecalis plasmid pAM.beta.1

L3 ANSWER 199 OF 209 CAPLUS COPYRIGHT 1997 ACS
TI Genetic aspects of the study of entomopathogenic bacteria

L3 ANSWER 200 OF 209 CAPLUS COPYRIGHT 1997 ACS
TI Specificities of monoclonal antibodies against the activated .delta.-endotoxin of Bacillus ***thuringiensis*** var. ***thuringiensis***

L3 ANSWER 201 OF 209 CAPLUS COPYRIGHT 1997 ACS
TI Expression of an enterobacterial gene for antibiotic resistance under control of regulatory signals of Bacillus ***thuringiensis*** in gram-negative and gram-positive bacteria

L3 ANSWER 202 OF 209 CAPLUS COPYRIGHT 1997 ACS
TI Transfer of Bacillus ***thuringiensis*** plasmids coding for .delta.-endotoxin among strains of B. ***thuringiensis*** and B. cereus

_3 ANSWER 203 OF 209 CAPLUS COPYRIGHT 1997 ACS
TI Cloning and expression of the crystal protein genes from Bacillus ***thuringiensis*** strain berliner 1715

_3 ANSWER 204 OF 209 CAPLUS COPYRIGHT 1997 ACS
TI Cloning and expression of promotor fragments of Bacillus ***thuringiensis*** DNA in Escherichia coli cells

_3 ANSWER 205 OF 209 CAPLUS COPYRIGHT 1997 ACS
TI Structure of cloned ribosomal DNA cistrons from Bacillus ***thuringiensis***

_3 ANSWER 206 OF 209 CAPLUS COPYRIGHT 1997 ACS
TI Antibacterial activity of zearalenone

_3 ANSWER 207 OF 209 CAPLUS COPYRIGHT 1997 ACS
TI Effect of mycotoxins separately and in mixtures with microbial and viral preparations on the survival rate, behavior, respiration, and the activity of several redox enzymes in Lepidopterae

_3 ANSWER 208 OF 209 CAPLUS COPYRIGHT 1997 ACS
TI Inhibitory effects of foliage extracts of some forest trees on commercial Bacillus ***thuringiensis***

_3 ANSWER 209 OF 209 CAPLUS COPYRIGHT 1997 ACS
TI Integrated control of muscid flies in poultry houses using predatormites, selected pesticides, and microbial agents

L3 ANSWER 12 OF 209 CAPLUS COPYRIGHT 1997 ACS
AN 1996:473391 CAPLUS DN 125:161101
TI Cross-resistance of the diamondback moth indicates altered interactions with domain II of Bacillus ***thuringiensis*** toxins
AU Tabashnik, Bruce E.; Malvar, Thomas; Liu, Yong-Baio; Finson, Naomi; Borthakur, Dulal; Shin, Byung-Sik; Park, Seung-Hwan; Masson, Luke; de Maagd, Ruud A.; Bosch, Dirk
CS Dep. Entomology, Univ. Hawaii, Honolulu, HI, 96822, USA
SO Appl. Environ. Microbiol. (1996), 62(8), 2839-2844 CODEN: AEMIDF; ISSN: 0099-2240 DT Journal LA English
AB We compared responses to six insecticidal crystal proteins from Bacillus ***thuringiensis*** by a Cry1A-resistant strain (NO-QA) and a susceptible strain (LAB-P) of the diamondback moth, Plutella xylostella. The resistant strain showed >100-fold cross-resistance to Cry1J and to H04, a ***hybrid*** with domains I and II of Cry1Ab and domain III of Cry1C. Cross-resistance was sixfold to Cry1Bb and threefold to Cry1D. The potency of Cry1I did not differ significantly between the resistant and susceptible strains. Cry2B did not kill resistant or susceptible larvae. By combining these new data with previously published results, we classified responses to 14 insecticidal crystal proteins by strains NO-QA and LAB-P. NO-QA showed high levels of resistance to Cry1Aa, Cry1Ab, and Cry1Ac and high levels of cross-resistance to Cry1F, Cry1J, and H04. Cross-resistance was low or nil to Cry1Ba, Cry1Bb, Cry1C, Cry1D Cry1I, and Cry2A. Cry1E and Cry2B showed little or no toxicity to susceptible or resistant larvae. In endrograms based on levels of amino acid sequence similarity among proteins, Cry1F and Cry1J clustered together with Cry1A proteins for domain II, but not for domain I or III. High levels of cross-resistance to Cry1Ab-Cry1C ***hybrid*** H04 show that although Cry1C is toxic to NO-QA, domain III or Cry1C is not sufficient to restore toxicity when it is combined with domains I and II of Cry1Ab. Thus, diamondback moth strain NO-AQ cross-resistance extends beyond the Cry1A family of proteins to at least two other families that exhibit high levels of amino sequence similarity with Cry1A in domain II (Cry1F and Cry1J) and to a protein that is identical to Cry1Ab in domain II (H04). The results of this study imply that resistance to Cry1A alters interactions between the insect and domain II.

L3 ANSWER 15 OF 209 CAPLUS COPYRIGHT 1997 ACS
AN 1996:377212 CAPLUS DN 125:51514
TI Novel strains of Bacillus that produce insecticidal proteins during vegetative growth and their genetic engineering
IN Warren, Gregory Wayne; Koziel, Michael Gene; Mullins, Martha Alice; Nye, Gordon James; Carr, Brian; Desai, Nalini Mano; Kostichka, Kristy; Duck, Nicholas Brendan; Estruch, Juan Jose
PA Ciba-Geigy A.-G., Switz.
SO PCT Int. Appl., 242 pp. CODEN: PIXXD2
PI WO 9610083 A1 960404
DS W: AM, AU, BB, BG, BR, BY, CA, CN, CZ, EE, FI, GE, HU, IS, JP, KG, KP, KR, KZ, LK, LR, LT, LV, MD, MG, MK, MN, MX, NO, NZ, PL, RO, RU, SG, SI, SK, TJ, TM, TT, UA, UZ, VN RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, DE, DK, ES, FR, GA, GB, GR, IE, IT, LU, MC, ML, MR, NE, NL, PT, SE, SN, TD, TG AI WO 95-EP3826 950927 PRAI US 94-314594 940928 US 95-463483 950605 DT Patent LA English
AB Bacillus strains capable of producing pesticidal proteins and auxiliary proteins during vegetative growth are described and the proteins are purified and genes encoding the proteins are cloned. The proteins and genes are useful in pest management programs (nodata). A Bacillus cereus isolate (strain AB78) that was significantly active against corn rootworm was isolated and characterized. Culture supernatants were very active against Western and Norther corn rootworms and had an overall spectrum of activity that was different from that of .delta.-endotoxins. Purifn. of the protein and cloning of the gene and raising of antibodies to the protein are described. Similar proteins were isolated from Bacillus ***thuringiensis*** strains AB88 and AB424 that were active against black cutworm (Agrotis ipsilon), Ostrinia nubilalis, and Spodoptera. Vegetative insecticidal protein (VIP) homologs and their genes were also isolated from Bacillus ***thuringiensis*** tenebrionis. Std. genetic techniques were used to express recombinant VIP proteins, ***fusion*** proteins contg. them, variants omitting the secretion signal peptide moieties or contg. ***fused*** vacuolar targeting signal peptides, and genes optimized for expression in maize.

L3 ANSWER 19 OF 209 CAPLUS COPYRIGHT 1997 ACS
AN 1996:290828 CAPLUS DN 124:335672
TI Lepidopteran pesticidal compositions comprising ***chimeric*** CryIF and CryIA(c) .delta.-endotoxins
IN Bradfisch, Gregory A.; Thompson, Mark; Schwab, George E.
PA Mycogen Corp., USA
SO U.S., 60 pp. CODEN: USXXAM
PI US 5508264 A 960416
AI US 94-349867 941206 DT Patent LA English
AB Compns. comprising ***chimeric*** combinations of CryIF ***chimeric*** and CryIA(c) Bacillus ***thuringiensis*** .delta.-endotoxin excellent activity against lepidopteran pests suchas the corn earworm Heliothis zea. Thus, a lactose-inducible Pseudomonas fluorescens strain comprising a gene encoding CryIF/CryIA(b) toxin, and P. fluorescens MR436, which comprises a gene encoding a CryIA(c)/CryIA(b) ***chimeric*** toxin, were constructed by std. recombinant DNA techniques. One such ***chimeric*** toxin has the full toxin portion of cryIF (amino acids 1-601) and a heterologous protoxin (amino acids 602 to the C-terminus) derived from a cryIA(b) or 436 toxin.

L3 ANSWER 20 OF 209 CAPLUS COPYRIGHT 1997 ACS
AN 1996:278656 CAPLUS DN 124:308923
TI Domain III substitution in Bacillus ***thuringiensis*** delta-endotoxin CryIA(b) results in superior toxicity for Spodoptera exigua and altered membrane protein recognition
AU de Maagd, Ruud A.; Kwa, Marcel S. G.; van der Klei, Hilde; Yamamoto, Takashi; Schipper, Bert; Vlak, Just M.; Stiekema, Willem J.; Bosch, Dirk
CS Dep. Mol. Biol., Cent. Plant Breeding Reprodn. Res., Wageningen, 6700 AA, Neth.
SO Appl. Environ. Microbiol. (1996), 62(5), 1537-1543 CODEN: AEMIDF; ISSN: 0099-2240 DT Journal LA English
AB To test our hypothesis that substitution of domain III of Bacillus ***thuringiensis*** delta-endotoxin (Cry) proteins might improve toxicity to pest insects, e.g., Spodoptera exigua, in vivo recombination was used to produce a no. of cryIA(b)-cryIC ***hybrid*** genes. A rapid screening assay was subsequently exploited to select ***hybrid*** genes encoding sol. protoxins. Screening of 120 recombinants yielded two different ***hybrid*** genes encoding sol. proteins with domains I and II of CryIA(b) and domain III of CryIC. These proteins differed by only one amino acid residue. Both ***hybrid*** protoxins gave a protease-resistant toxin upon in vitro activation by trypsin. Bioassays showed that one of these CryIA(b)-CryIC ***hybrid*** proteins (H04) was highly toxic to S. exigua compared with the parental CryIA(b) proteins and significantly more toxic than CryIC. In semiquant. binding studies with biotin-labeled toxins and intact brush border membrane vesicles of S. exigua, this domain III substitution appeared not to affect binding-site specificity. However, binding to a 200-kDa protein by CryIA(b) in prepns. of solubilized and blotted brush border membrane vesicle proteins was completely abolished by the domain III substitution. A reciprocal ***hybrid*** contg. domains I and II of CryIC and domain III of CryIA(b) did bind to the 200-kDa protein, confirming that domain III of CryIA(b) was essential for this reaction. This results show that domain III of CryIC protein plays an important role in the level of toxicity to S.

exigua, that substitution of domain III may be apowerful tool to increase the repertoire of available active toxins for pest insects, and that domain III is involved in binding to gut epithelium membrane proteins of S. exigua.

L3 ANSWER 24 OF 209 CAPLUS COPYRIGHT 1997 ACS
AN 1996:117237 CAPLUS DN 124:166737
TI Comparative study in three systems of heterologous expression of recombinant .delta.-endotoxins from Bacillus ***thuringiensis*** in Escherichia coli
AU Vazquez, Roberto; Prieto, Dmitri; Oloriz, Maria Ileana; De La Riva, Gustavo A.; Sleman-Housein, Guillermo
CS Div. Agricultura, Centro Ingenieria Genetica Biotecnol., Havana, 10600, Cuba
SO Rev. Latinoam. Microbiol. (1995), 37(3), 237-44 CODEN: RLMIAA; ISSN: 0187-4640 DT Journal LA Spanish
AB The crylA(b) and crylA(c) genes encoding active fragments of Bacillus ***thuringiensis*** .delta.-endotoxins were cloned downstream of the pR and pT7 promoters from the .lambda. and T7 bacteriophages, resp. The crylA(b) gene was also ***fused*** with the gene encoding protein A from Staphylococcus aureus cloned under the control of the pR promoter. There were no remarkable differences in the expression levels of the cloned genes in E. coli, but the Western blot anal. allowed distinct protein quality for the three expression systems. The best expression model for the prodn. of .delta.-endotoxin toxic fragments in E. coli is the one based on .lambda. pR promoter.

L3 ANSWER 32 OF 209 CAPLUS COPYRIGHT 1997 ACS
AN 1995:916027 CAPLUS DN 124:2823
TI Domain III exchanges of Bacillus ***thuringiensis*** cryla toxins affect binding to different gypsy moth midgut receptors
AU Lee, Mi Kyong; Young, B. A.; Dean, D. H.
CS Dep. Biochem., Ohio State Univ., Columbus, OH, 43210, USA
SO Biochem. Biophys. Res. Commun. (1995), 216(1), 306-12 CODEN: BBRCA9; ISSN: 0006-291X DT Journal LA English
AB Aminopeptidase-N, purified from gypsy moth (Lymantria dispar L.) brush border membrane vesicles, exhibited specific binding to CrylAc toxin but not to CrylAa toxin. CrylAa-CrylAc ***hybrid*** toxins were used to localize the aminopeptidase-N binding region on CrylAc. Slot blot assays and ligand blot expts. demonstrated that the ***hybrid*** toxins which have the residues 451 to 623, comprising essentially domain III, from CrylAc toxin exhibited strong binding to purified aminopeptidase-N and 120 kDa brush border membrane protein. In contrast, the ***hybrid*** toxins which have the residues 451 to 623 from CrylAa toxin failed to bind to aminopeptidase-N, but did bind to another receptor, a 210 kDa protein. This is the first direct evidence that domain III is involved in receptor binding and the first to demonstrate that domain III substitutions direct the binding of these toxins to different gypsy moth midgut receptors.

L3 ANSWER 33 OF 209 CAPLUS COPYRIGHT 1997 ACS
AN 1995:712100 CAPLUS DN 123:249035
TI ***Hybrid*** toxins of Bacillus ***thuringiensis***
IN Bosch, Hendrik Jan; Stiekema, Willem Johannes
PA Sandoz Ltd., Switz.; Sandoz-Patent-GmbH; Sandoz-Erfindungen Verwaltungsgesellschaft mbH
SO PCT Int. Appl., 65 pp. CODEN: PIXXD2
PI WO 9506730 A1 950309
DS W: AU, BR, CA, CZ, HU, JP, KR, PL, RU, SK, US RW: AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE AI WO 94-EP2909 940901 PRAI GB 93-18207 930902 DT Patent LA English
AB A ***hybrid*** toxin of Bacillus ***thuringiensis*** is provided, which ***hybrid*** toxin is comprised of a C-terminal domain III of a 1st cry gene (e.g. crylC) and an N-terminal domain of a 2nd cry protein. Construction of ***hybrid*** toxins of crylA/crylC and crylE/crylC of B. ***thuringiensis*** was shown. The N-terminal domain may also be selected from other cry proteins such as crylA(a), crylA(b), crylA(c), etc.

L3 ANSWER 34 OF 209 CAPLUS COPYRIGHT 1997 ACS
AN 1995:696054 CAPLUS DN 123:77175
TI Insecticidal proteins constructed from Bacillus ***thuringiensis*** .delta.-endotoxin and Androctonus australis neurotoxin AaHIT
IN Ely, Susan
PA Zeneca Ltd., UK
SO PCT Int. Appl., 27 pp. CODEN: PIXXD2
PI WO 9511305 A2 950427
DS W: AU, BB, BG, BR, BY, CA, CN, CZ, FI, GE, HU, JP, KG, KP, KR, KZ, LK, LT, LV, MD, MG, MN, NO, NZ, PL, RO, RU, SI, SK, TJ, TT, UA, US, UZ, VN
RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, DE, DK, ES, FR, GA, GB, GR, IE, IT, LU, MC, ML, MR, NE, NL, PT, SE, SN, TD, TG AI WO 94-GB2274 941018 PRAI GB 93-21469 931018 DT Patent LA English
AB ***Chimeric*** insecticidal proteins comprise at least part of a Bacillus ***thuringiensis*** .delta.-endotoxin ***fused*** to a venom-derived insecticidal protein, such as the AaHIT peptide obtainable from Androctonus australis Hector. The .delta.-endotoxin portion protects the venom-derived protein and delivers it to the insect gut. DNA constructs encoding such ***chimeric*** proteins may be used to express said proteins in biol. organisms. Exposure of insects to the ***chimeric*** insecticidal proteins is achieved through application to plants of an insecticidal compn. contg. said proteins or through expression of said proteins within transgenic plants. Thus, the neurotoxin AaHIT gene from A. australis Hector was modified to optimize expression in Escherichia coli or dicotyledonous plants and to introduce unique restriction sites into the gene or flanking regions. Further, a trypsin-cleavage site was created within the ***chimeric*** protein to allow release of the AaHIT protein moiety into the insect gut. This synthetic gene was in-frame ***fused*** to the gene coding for the N-terminal portion of CrylA(c), CryV, or CrylIIA.delta.-endotoxin.

L3 ANSWER 46 OF 209 CAPLUS COPYRIGHT 1997 ACS
AN 1995:220383 CAPLUS DN 122:25850
TI Insecticidal ***fusion*** proteins of Bacillus ***thuringiensis*** var. kurstaki HD-1
IN Akashi, Akira; Oomori, Iwao
PA Toa Gosei Chem Ind, Japan
SO Jpn. Kokai Tokkyo Koho, 11 pp. CODEN: JKXXAF
PI JP 06192295 A2 940712 Heisei
AI JP 91-59504 910301 DT Patent LA Japanese
AB An insecticidal ***fusion*** protein of Bacillus ***thuringiensis*** var. kurstaki HD-1 is prepd. by substitution of the C-terminus of gene cry-1-2 protein with the C-terminus of gene cry-1-1 protein. The ***fusion*** protein exhibits improved resistance to proteinase. Prepn. of the ***fusion*** protein in transgenic Bacillus subtilis and characterization of the product were also shown.

L3 ANSWER 47 OF 209 CAPLUS COPYRIGHT 1997 ACS
AN 1995:206457 CAPLUS DN 122:25829
TI Insect resistance of transgenic plants that express modified Bacillus ***thuringiensis*** crylA(b) and crylC genes: a resistance management strategy
AU van der Salm, Theo; Bosch, Dirk; Honee, Guy; Feng, Lanxiang; Munsterman, Ellie; Bakker, Petra; Stiekema, Willem J.; Visser, Bert
CS Dep. Molecular Biology, DLO-Centre Plant Breeding Reproduction Res., Wageningen, 6700 AA, Neth.
SO Plant Mol. Biol. (1994), 26(1), 51-9 CODEN: PMBIDB; ISSN: 0167-4412 DT Journal LA English
AB Tobacco and tomato plants were generated exhibiting insect resistance due to the introduction of modified crylA(b) and crylC genes of Bacillus ***thuringiensis*** . Limited modifications at selected regions of the coding sequences of both genes are sufficient to obtain resistance against Spodoptera exigua, Heliothis virescens and Manduca sexta. The criteria used to modify both genes demonstrate that the removal of sequence motifs potentially resulting in premature polyadenylation and transcript instability causes increased insect resistance. The expression of a crylC-crylA(b) ***fusion*** resulting in protection against S. exigua, H. virescens and M. sexta demonstrates the potential of expressing translational ***fusions*** , not only to broaden the insect resistance of transgenic plants, but also to simultaneously employ different gene classes in resistance management strategies.

L3 ANSWER 52 OF 209 CAPLUS COPYRIGHT 1997 ACS
AN 1994:573019 CAPLUS DN 121:173019

TI Location of a lepidopteran specificity region in insecticidal crystal protein CryIIA from Bacillus ***thuringiensis***
AU Liang, Y.; Dean, D. H.
CS Dep. Biochem., Ohio State Univ., Columbus, OH, 43210, USA
SO Mol. Microbiol. (1994), 13(4), 569-75 CODEN: MOMIEE; ISSN: 0950-382X DT Journal LA English
AB The Bacillus ***thuringiensis*** insecticidal crystal protein CryIIA has both high mosquito activity and gypsy moth activity; in contrast CryIIB, which is 87% homologous, displays no mosquitoactivity and has a three-fold lower gypsy moth activity. The regions responsible for specificity against gypsy moth (Lymantria dispar) and mosquito (Aedes aegypti) larvae were located by introducing MluI and XhoI sites into homologous positions within the putative domain II of both CryIIA and CryIIB genes, which divided almost equally the resp. second domains into three regions. Taking advantage of naturally occurring NheI and NarI sites that border the putative domain II, a set of seven ***chimeric*** proteins were produced by exchanging all combinations of those regions between CryIIA and CryIIB. Anal. of the toxicity of these ***chimeric*** proteins demonstrated that the lepidopteran and dipteran specificity regions of CryIIA were not colinear. While the specificity region of CryIIA against mosquito larvae involved region 1 and probably also region 2, the specificity region of CryIIA against gypsy moth larvae was located within region 2.

L3 ANSWER 54 OF 209 CAPLUS COPYRIGHT 1997 ACS
AN 1994:571754 CAPLUS DN 121:171754
TI Cloning of a new cryIA(a) gene from Bacillus ***thuringiensis*** strain FU-2-7 and analysis of ***chimeric*** CryIA(a) proteins for toxicity
AU Udayasuriyan, Varatharajalu; Nakamura, Akira; Mori, Hironori; Masaki, Haruhiko; Uozumi, Takeshi
CS Fac. Agric., Univ. Tokyo, Tokyo, 113, Japan
SO Biosci., Biotechnol., Biochem. (1994), 58(5), 830-5 CODEN: BBBIEJ; ISSN: 0916-8451 DT Journal LA English
AB The authors cloned the cryIA(a) gene from Bacillus ***thuringiensis*** strain FU-2-7, one of the toxin genes encoding lepidopteran-specific protoxins. Sequences anal. of the gene showed two amino acid differences (Pro77 to Leu and Phe965 to Ser) from the CryIA(a) of B. ***thuringiensis*** strain HD-1. The authors constructed ***chimeric*** cryIA(a) genes using FU-2-7 and HD-1 cryIA(a) genes and isolated the ***chimeric*** protoxins, as well as the parental ones, from Escherichia coli cells harboring the recombinant plasmids to examine the effects of the two amino acid variations on the toxicity. FU-2-7 CryIA(a) protein was about half as toxic against the smaller tea tortrix, Adoxophyes sp., and one-third as toxic against the silkworm, Bombyx mori, as that of HD-1. On the other hand, a ***chimeric*** CryIA(a) protein with a single replacement of Phe965 to Ser had nearly the same toxicity as the HD-1 CryIA(a) against the smaller tea tortrix and one-third the toxicity against silkworm as that of HD-1. This improved property of the ***chimeric*** CryIA(a) protoxin may be useful for widening its application to crop protection in sericultural countries.

L3 ANSWER 66 OF 209 CAPLUS COPYRIGHT 1997 ACS
AN 1994:155282 CAPLUS DN 120:155282
TI Primary structure of cryX, the novel .delta.-endotoxin-related gene from Bacillus ***thuringiensis*** spp. galleriae
AU Shevelev, A. B.; Svarinsky, M. A.; Karasin, A. I.; Kogan, Ya. N.; Chestukhina, G. G.; Stepanov, V. M.
CS Institute of Microbial Genetics (VNIIGenetika), Laboratory of Protein Chemistry, 1st Dorozhny 1, Moscow, 113545, Russia
SO FEBS Lett. (1993), 336(1), 79-82 CODEN: FEBLAL; ISSN: 0014-5793 DT Journal LA English
AB A cry-related sequence, designated cryX (EMBL X75019), was localized upstream of cryIG, the .delta.-endotoxin gene cloned from Bacillus ***thuringiensis*** galleriae and sequenced earlier (Smulevitch, S. V., et al., 1991). Anal. of the cryX complete nucleotide sequence enabled the authors to explain its virtual crypticity and to reveal the ***chimeric*** structure of the genes, cryX and cryIG. The amino acid sequence of 1151 residues encoded by the continuous reading frame of cryX is similar to the other .delta.-endotoxins but differs essentially from them.

L3 ANSWER 72 OF 209 CAPLUS COPYRIGHT 1997 ACS
AN 1993:663924 CAPLUS DN 119:263924
TI Construction of a gene for a ***hybrid*** protein based on Bacillus ***thuringiensis*** .delta.-endotoxin CryIA(a) and CryIIIA sequences and expression of its derivatives in Escherichia coli
AU Shadenkov, A. A.; Kadyrov, R. M.; Uzbekova, S. V.; Kuzmin, E. V.; Osterman, A. L.; Chestukhina, G. G.; Shemyakin, M. F.
CS All-Russian Res. Inst. Agric. Biotechnol., Moscow, 127253, Russia
SO Mol. Biol. (Moscow) (1993), 27(4), 952-9 CODEN: MOBIBO; ISSN: 0026-8984 DT Journal LA Russian
AB The gene encoding the 5'-terminal fragment (codons 1-565) of the Bacillus ***thuringiensis*** tenebrionis .delta.-endotoxin CryIIIA, specific for Coleoptera, was cloned. This sequence was extended with either a homologous fragment of CryIA(a) from B. t. kurstaki HD-1 or the homologous fragment together with in-frame coding sequences for kanamycin phosphotransferase (NPTII) or .beta.-glucuronidase (GUS). Gene derivs. obtained were expressed in Escherichia coli. Anal. of ***hybrid*** polypeptides confirmed the enzymic activities of bifunctional proteins and demonstrated the toxic properties of the ***fusion*** toxin-NPTII against the Colorado potato beetle (Leptinotarsa decemlineata).

L3 ANSWER 82 OF 209 CAPLUS COPYRIGHT 1997 ACS
AN 1993:422255 CAPLUS DN 119:22255
TI Synthetic genes for delta-endotoxins optimized for expression in maize
IN Koziel, Michael G.; Desai, Nalini M.; Lewis, Kelly S.; Kramer, Vance C.; Warren, Gregory W.; Evola, Stephen V.; Crossland, Lyle D.; Wright, Martha S.; Merlin, Ellis J.; et al.
PA Ciba-Geigy A.-G., Switz.
SO PCT Int. Appl., 289 pp. CODEN: PIXXD2
PI WO 9307278 A1 930415
DS W: AU, BB, BG, BR, CA, CS, FI, HU, JP, KP, KR, LK, MG, MN, MW, NO, PL, RO, RU, SD, US
RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, DE, DK, ES, FR, GA, GB, GR, IE, IT, LU, MC, ML, MR, NL, SE, SN, TD, TG AI WO 92-US8476 921005 PRAI US 91-772027 911004 US 92-951715 920925 DT Patent LA English
AB Synthetic genes encoding Bacillus ***thuringiensis*** .delta.-endotoxins with codon usage optimized for expression in maize are constructed. When the genes are expressed in maize, the toxins protect the plants from Lepidopteran or Coleopteran insects. Synthetic genes encoding CryIA(b) proteins or heat-stable CryIA(b) proteins were prepd. and expressed in maize. Expression levels were increased 1,000- to 20,0000-fold (relative to unaltered genes). The promoters from a pith-specific tryptophan synthase subunit gene and a pollen-specific Ca2+-dependent protein kinase gene were used to drive tissue-specific expression of these genes. Tissue-specific expression of modified toxin genes ***fused*** to these promoters were demonstrated in maize.

L3 ANSWER 83 OF 209 CAPLUS COPYRIGHT 1997 ACS
AN 1993:403014 CAPLUS DN 119:3014
TI Field performance of elite transgenic maize plants expressing an insecticidal protein derived from Bacillus ***thuringiensis***
AU Koziel, Michael G.; Beland, Gary L.; Bowman, Cindy; Carozzi, Nadine B.; Crenshaw, Rebecca; Crossland, Lyle; Dawson, John; Desai, Nalini; Hill, Martha
CS Agric. Biotechnol. Res. Unit, Ciba-Geigy, Research Triangle Park, NC, 27709, USA
SO Bio/Technology (1993), 11(2), 194-200 CODEN: BTCHDA; ISSN: 0733-222X DT Journal LA English
AB A synthetic gene encoding a truncated version of the CryIA(b) protein derived from B. ***thuringiensis*** was introduced into immature embryos of an elite line of maize using microprojectile bombardment. This gene was expressed using either the CaMV 35S promoter or a combination of 2 tissue specific promoters derived from maize. High levels of CryIA(b) protein were obtained using both promoter configurations. ***Hybrid*** maize plants resulting from crosses of transgenic elite inbred plants with com. inbred lines were evaluated for resistance to European corn borer under field conditions. Plants expressing high levels of the insecticidal protein exhibited excellent resistance to repeated heavy infestations of this pest.

L3 ANSWER 85 OF 209 CAPLUS COPYRIGHT 1997 ACS
AN 1993:161915 CAPLUS DN 118:161915
TI Expression of mutated .delta.-endotoxin gene of Bacillus ***thuringiensis*** subsp. tenebrionis in E. coli and insecticidal activity against Coleopteran insects
AU Rhim, Seong Lyul
CS Dep. Genet. Eng., Hallym Univ., Chuncheon, 200-702, S. Korea
SO Mol. Cells (1992), 2(2), 207-11 CODEN: MOCEEK; ISSN: 1016-8478 DT Journal LA English
AB A cloned .delta.-endotoxin gene from Bacillus ***thuringiensis*** subsp. tenebrionis (Btt) was mutated at 5'-end region by site directed mutagenesis. The mutation results in creation of a new BamHI restriction site. For general cloning and further researches such as as anal. of gene expression, the promoter region was replaced with a synthesized oligonucleotide contg. SmaI, BglII and

BamHI restriction sites. In the synthesized sequence, a ATG-start codon was included before the new BamHI site. This sequence was subsequently ***fused*** to LacZ'-promoter. The expression of two proteins indicated a second ribosome binding site of the toxin encoding sequence. It was found by the Western blot analyses that the expression of intact and modified Btt-toxin genes showed no significant differences in E. coli. Furthermore, biotest with ext. of E. coli transformant by mutated Btt-toxin gene showed toxin activity against coleopteran insect larvae.

L3 ANSWER 88 OF 209 CAPLUS COPYRIGHT 1997 ACS
AN 1993:117706 CAPLUS DN 118:117706
TI Expression of a ***hybrid*** gene for bifunctional insect toxin-glucuronidase protein in transgenic tobacco
AU Shchabenkov, A. A.; Uzbekova, S. V.; Kuz'min, E. V.; Zolotova, T. B.; Eisner, G. I.; Shemyakin, M. F.
CS Nauchno-Issled. Inst. S-Kh. Biotekhnol., Moscow, Russia
SO Dokl. Akad. Nauk (1992), 325(1), 183-6, 1 plate [Biochem.] CODEN: DAKNEQ DT Journal LA Russian
AB The crylA(a) gene region coding for the Bacillus ***thuringiensis*** kurstaki .delta. endotoxin active fragment was ***fused*** in frame to a bacterial marker .beta.-glucuronidase gene to express the N-terminus active endodoxin-C-terminus glucuronidase protein in transgenic tobacco. Plant cells contg. glucuronidase activity were screened for the presence of ***fused*** protein. Proteolysis released the endotoxin. Transgenic plants were demonstrated to be resistant to Lymantria dispar moth and second and hird instar larvae.

L3 ANSWER 90 OF 209 CAPLUS COPYRIGHT 1997 ACS
AN 1993:95291 CAPLUS DN 118:95291
TI Expression of a ***chimeric*** CaMV 35S Bacillus ***thuringiensis*** insecticidal protein gene in transgenic tobacco. [Erratum to document cited in CA118(3):17151c]
AU Carozzi, Nadine B.; Warren, Gregory W.; Desai, Nalini; Jayne, Susan M.; Lotstein, Richard; Rice, Douglas A.; Evola, Stephen; Koziel, Michael G.
CS Ciba-Geigy Agric. Biotechnol. Res. Unit, Research Triangle Park, NC, 27709, USA
SO Plant Mol. Biol. (1993), 21(2), 413 CODEN: PMBIDB; ISSN: 0167-4412 DT Journal LA English
AB An error in ref. 27 has been cor. The error was not reflected in the abstr. or the index entries.

L3 ANSWER 92 OF 209 CAPLUS COPYRIGHT 1997 ACS
AN 1993:54314 CAPLUS DN 118:54314
TI Suppression of protein structure destabilizing mutations in Bacillus ***thuringiensis*** .delta.-endotoxins by second site mutations
AU Almond, Brian D.; Dean, Donald H.
CS Dep. Mol. Genet., Ohio State Univ., Columbus, OH, 43210, USA
SO Biochemistry (1993), 32(4), 1040-6 CODEN: BICHAW; ISSN: 0006-2960 DT Journal LA English OS CJACS-IMAGE; CJACS
AB Reciprocal exchange of a small region (residues 429-450) within the specificity detg. region of 2 B. ***thuringiensis*** .delta.-endotoxins, CrylAa and CrylAc, resulted in 2 recombinant proteins that possess a decreased insecticidal activity to Bombyx mori and Manduca sexta. Site-directed mutations introduced in this region of 1 of the recombinant proteins, for restoring insecticidal activity, resulted in further redn. of toxicity. The loss of insecticidal activity in the mutants and the original recombinants was assocd. with altered toxin protein structure, as measured by sensitivity to intracellular and exogenous proteases. The structural instability of the site-directed mutant proteins could be suppressed genetically by subcloning the mutated region into crylAc or by introducing second site mutations in defined regions of the mutated crylAa gene. The second site mutations, by themselves, also produced unstable proteins. Thus, this small region does not suffice as a specificity detg. region for M. sexta.

L3 ANSWER 94 OF 209 CAPLUS COPYRIGHT 1997 ACS
AN 1993:17151 CAPLUS DN 118:17151
TI Expression of a ***chimeric*** CaMV 35S Bacillus ***thuringiensis*** insecticidal protein gene in transgenic tobacco
AU Carozzi, Nadine B.; Warren, Gregory W.; Desai, Nalini; Jayne, Susan M.; Lotstein, Richard; Rice, Douglas A.; Evola, Stephen; Koziel, Michael G.
CS Ciba-Geigy Agric. Biotechnol. Res. Unit, Research Triangle Park, NC, 27709, USA
SO Plant Mol. Biol. (1992), 20(3), 539-48 CODEN: PMBIDB; ISSN: 0167-4412 DT Journal LA English
AB Insecticidal transgenic tobacco plants contg. a truncated B. ***thuringiensis*** crylA(b) crystal protein (ICP) gene expressed from the CaMV 35S promoter were analyzed for ICP gene expression under field and greenhouse conditions over the course of a growing season. Information on temporal and tissue-specific expression of a CaMV 35S/crylA(b) gene is presented. Levels of crylA(b) protein and mRNA were compared in both homozygous and hemizygous lines throughout plant development. Levels of ICP mRNA and protein increased during plant development with a pronounced rise in expression at the time of flowering. Homozygous ICP lines produced higher levels of ICP than did the corresponding hemizygous lines. ELISA anal. of different tissues in the tobacco plant showed ICP gene expression in most tissues with a predominance of ICP in older tissue. All transgenic ICP tobacco lines which were studied in the field and greenhouse contained 400 ng to 1 .mu.g ICP per g fresh wt. in leaves from the mid-section of the plant at flowering. The amts.of ICP produced by field lines were directly comparable to levels obsd. in greenhouse-grown plants.

L3 ANSWER 101 OF 209 CAPLUS COPYRIGHT 1997 ACS
AN 1992:442755 CAPLUS DN 117:42755
TI Extending the host range of insecticidal proteins using peptides that bind gut cells
IN Sivasubramanian, Natarajan; Federici, Brian A.
PA University of California, Oakland, USA
SO PCT Int. Appl., 97 pp. CODEN: PIXXD2
PI WO 9117254 A1 911114 DS W: AU, CA, JP, KR
RW: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LU, NL, SE AI WO 91-US3008 910502 PRAI US 90-518575 900503 DT Patent LA English
AB The host range of insecticidal proteins such as .delta.-endotoxins is exteneded by ***fusing*** with a peptide that binds a receptor in the gut wall to the protein. ***Chimeric*** genes for ***fusion*** proteins of Bacillus ***thuringiensis*** tenebrionis .delta.-endotoxin and the gp64 protein of Autographa californica multiple nuclear polyhedrosis virus were constructed by std. methods and expressed in Escherichia coli from bacteriophage T7 promoter. The ***fusion*** protein accumulated as inclusion bodies. Lima beans coated with cells expressing these genes were used as feed for Trichoplusia ni larvae. Larvae fed on this showed damage to the midgut.

L3 ANSWER 106 OF 209 CAPLUS COPYRIGHT 1997 ACS
AN 1992:124512 CAPLUS DN 116:124512
TI The C-terminal domain of the toxic fragment of a Bacillus ***thuringiensis*** crystal protein determines receptor binding
AU Honee, G.; Convents, D.; Van Rie, J.; Jansens, S.; Peferoen, M.; Visser, B.
CS Cent. Plant Breed. Reprod. Res., Wageningen, 6700 AA, Neth.
SO Mol. Microbiol. (1991), 5(11), 2799-806 CODEN: MOMIEE; ISSN: 0950-382X DT Journal LA English
AB The insecticidal crystal proteins of B. ***thuringiensis*** show a high degree of specificity. In vitro binding studies with several crystal proteins demonstrated a correlation between toxicity and binding to receptors of larval midgut epithelial cells. To study the domain-function relationships of the toxic fragment, ***hybrid*** crystal proteins based on CrylA(b) and CrylC were constructed. Two out of 11 ***hybrid*** proteins constructed exhibited insecticidal activity. Both displayed an insecticidal spectrum similar to that of the parental crystal protein from which the C-terminal part of the toxic fragment originated. In addn., in vitro binding studies directly demonstrated the involvement of the C-terminal part of the toxic fragment in receptor binding. These results demonstrate that the C-terminal part of the toxic fragment dets. specific receptor binding, which in turn dets., to a large extent, the insect specificity.

L3 ANSWER 107 OF 209 CAPLUS COPYRIGHT 1997 ACS
AN 1992:77477 CAPLUS DN 116:77477
TI Construction of genes for bifunctional derivatives of Bacillus ***thuringiensis*** var. kurstaki insect toxin for expression in transgenic plants
AU Kuz'min, E. V.; Shadenkov, A. A.; Uzbekova, S. V.; Shemyakin, M. F.
CS Vses. Nauchno-Issled. Inst. S-kh. Biotekhnol., Moscow, USSR

SO Dokl. Akad. Nauk SSSR (1991), 321(2), 412-15, 1 plate [Biochem.] CODEN: DANKAS; ISSN: 0002-3264 DT Journal LA Russian
AB A plasmid, pRT103tt, was constructed with the toxin domain of .delta.-endotoxin gene of B. ***thuringiensis*** kurstaki under the control of the cauliflower mosaic virus (CaMV) 35 S promoter, the poly(A) signal from gene VI of CaMV, and a consensus translation initiation region. Plasmid pRT103tg and plasmid pRT103tn were constructed by ***fusing*** the gene for .beta.-glucuronidase or kanamycin phosphotransferase, resp., to the 3' end of the toxin domain reading frame on plasmid pRT103tt. To test the functionality of the proteins encoded by these vectors by expressing them in Escherichia coli, a SalI-NcoI fragment of expression vector pKK233-2 carrying the Ptrc promoter and the Shine-Delgarno sequence was inserted into these plasmids between the coding region and the 35S promoter. The toxin-.beta.-glucuronidase and the toxin-kanamycin phosphotransferase ***fusion*** proteins expressed by E. coli were 155 kDa and 90 kDa, resp., and showed the appropriate enzymic activity. The toxin domain protein and the toxin-kanamycin phosphotransferase ***fusion*** protein had insecticidal activity against Lymantria dispar, similar to a control B. ***thuringiensis*** kurstaki .delta.-endotoxin expressed in E. coli; the toxin-.beta.-glucuronidase ***fusion*** protein had lower insecticidal activity. The potential use of these vectors to transform plants is discussed.

L3 ANSWER 109 OF 209 CAPLUS COPYRIGHT 1997 ACS
AN 1992:77436 CAPLUS DN 116:77436
TI Isolation and cloning of Bacillus ***thuringiensis*** var Kurstaki HD73 toxin gene and construction of a ***chimeric*** gene for expression in plants.
AU Basu, Debabrata; Das, Sampa; Bandyopadhyay, Durba; Sen, S. K.
CS Bose Inst., Calcutta, 700 054, India
SO Indian J. Exp. Biol. (1991), 29(11), 1002-9 CODEN: IJEBA6; ISSN: 0019-5189 DT Journal LA English
AB B. ***thuringiensis*** Kurstaki HD73 crystal protein coded by gene CryIA(c)73 has been found to be sufficiently effective against the major pests of jute and chickpea. An attempt to isolate the gene and construct a ***chimeric*** gene for expression in plants was carried out. The plasmid CryIA(c)73 gene was cloned and tailored at the 3' end. The expression of the truncated gene was monitored in the minicell systems of Escherichia coli. The entomocidal property was found to be fully retained by the gene product. Deletion of the nucleotides at the 5' end was carried out and a ***chimeric*** gene construct of cryIA(c)73 was made in such a way that it was ***fused*** in frame with the GUS gene under the control of the CaMV 35S promoter with Nos polyadenylated terminus. Such a ***chimeric*** gene construct was used as the passenger of a Ti plasmid derived plant vector with kanamycin gene (NPTII) as the addnl. plant marker. Transformation through infection of tobacco and mustard plant cells in culture was carried out. Plants regenerated from the transformed cells showed the presence of gene GUS indicating the expression of the cloned ***fused*** gene. Also, Northern anal. established the presence of CryIA(c)73 gene transcripts in the transgenic plants.

L3 ANSWER 111 OF 209 CAPLUS COPYRIGHT 1997 ACS
AN 1991:672693 CAPLUS DN 115:272693
TI A temperature-stable Bacillus ***thuringiensis*** .delta.-endotoxin analog
IN Geiser, Martin; Moser, Jacqueline
PA Ciba-Geigy A.-G., Switz.
SO Eur. Pat. Appl., 41 pp. CODEN: EPXXDW
PI EP 440581 A1 910807
DS R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE AI EP 91-810050 910122 PRAI CH 90-302 900131 DT Patent LA German
AB A deriv. of Bacillus ***thuringiensis*** .delta.-endotoxin that is stable at >25.degree. is prepd. by expression of the cloned gene in Bacillus. The modified protein has a deletion of 26 amino acids starting at position 794 of the protein and a no. of C-terminal region substitutions resulting from substitution of the 3'-end of the CryIA(b) gene with a sequence from the cryIA(c) gene. The corresponding DNA was constructed by std. methods and introduced into a B. ***thuringiensis*** cryB. The .delta.-endotoxin content of spore suspensions from cultures grown at 25.degree. was 14.8 and 17.1 .mu.g toxin/mL for strains carrying control and novel deriv. genes, resp. When grown at 33.degree. the levels were 0.53and 17.6, resp.

L3 ANSWER 115 OF 209 CAPLUS COPYRIGHT 1997 ACS
AN 1991:552383 CAPLUS DN 115:152383
TI Generation of functional Bacillus ***thuringiensis*** toxin ***hybrid*** genes by in vivo recombination
AU Caramori, T.; Albertini, A. M.; Galizzi, A.
CS Dip. Genet. Microbiol. "A. Buzzati Traverso", Univ. Pavia, Italy
SO Genet. Biotechnol. Bacilli, [Proc. Int. Conf. Bacilli], 5th (1990), Meeting Date 1989, 191-9. Editor(s): Zukowski, Mark M.; Ganesan, A. T.; Hoch, James A. Publisher: Academic, San Diego, Calif. CODEN: 57DZAY DT Conference LA English
AB Eight different recombinant toxins were prepd. from the parasporal crystal genes of Bacillus ***thuringiensis*** . Plasmid vectors (pT173 and pGEM-173) were constructed to contain (1) the promoter region and roughly the first half of gene cryIA(a) from strain HDI-Dipel in one plasmid and (2) the 3' part of gene cryIA(c) from stain HD-73. The 2 sequences had in common .apprx.700 base pairs, corresponding to most of the variable region, and Escherichia coli transformants contg. the constructs all arose from a single recombination event.

L3 ANSWER 116 OF 209 CAPLUS COPYRIGHT 1997 ACS
AN 1991:552042 CAPLUS DN 115:152042
TI In vivo generation of hybrids between two Bacillus ***thuringiensis*** insect-toxin-encoding genes
AU Caramori, T.; Albertini, A. M.; Galizzi, A.
CS Dip. Genet. Microbiol. 'A. Buzzati-Traverso', Univ. Pavia, Pavia, 27100, Italy
SO Gene (1991), 98(1), 37-44 CODEN: GENED6; ISSN: 0378-1119 DT Journal LA English
AB The parasporal crystal of B. ***thuringiensis*** is composed of polypeptides highly toxic to a no. of insect larvae. The structural genes (cryIA) encoding the Lepidoptera-specific toxin from different bacterial strains diverge primarily in a single hypervariable region, whereas the N-terminal and C-terminal parts of the proteins are highly conserved. This report describes the generation of ***hybrid*** genes between two cryIA genes. Two truncated cryIA genes were cloned in a plasmid vector in such way as to have only the hypervariable region in common. The two truncated cryIA genes were sepd. by the tetracycline-resistance determinant (or part of it). In vivo recombination between the hypervariable regions of the cryIA genes reconstituted an entire ***hybrid*** cryIA gene. Direct sequence anal. of 17 recombinant plasmids identified eleven different crossover regions which did not alter the reading frame and allowed the prodn. of eight different ***hybrid*** proteins. The recombination events were independent from the RecA function of Escherichia coli. Some of the ***hybrid*** gene products were more specific in their insecticidal action and one had acquired a new biol. activity.

L3 ANSWER 121 OF 209 CAPLUS COPYRIGHT 1997 ACS
AN 1991:402524 CAPLUS DN 115:2524
TI New functional Bacillus ***thuringiensis*** .delta.-endotoxin ***hybrid*** genes obtained by in vivo recombination
IN Galizzi, Alessandro; Albertini, Alessandra; Caramori, Tiziana; Degrassi, Giuliano; Persic, Lidija
PA CRC Compagnia di Ricerca Chimica S.p.A., Italy
SO PCT Int. Appl., 64 pp. CODEN: PIXXD2
PI WO 9101087 A1 910207
DS W: AU, BR, JP, SU, US RW: AT, BE, CH, DE, DK, ES, FR, GB, IT, LU, NL, SE AI WO 90-EP1145 900712 PRAI IT 89-21243 890720 DT Patent LA English
AB B. ***thuringiensis*** .delta.-endotoxins with altered hypervariable regions are produced from ***hybrid*** genes obtained by in vivo recombination of genes encoding 2 different .delta.-endotoxins. These ***hybrid*** proteins may have altered insecticidal activities (no data). A plasmid contg. the 5' portion of the HD1 Dipel gene (including the hypervariable coding region) linked to the 3' portion of the HD73 gene (including the hypervariable coding region) with the tetracycline resistance (tetR) gene and contg. a chloramphenicol resistance (Cmr) gene was constructed. Escherichia coli (recA+ or recA-) were transformed with this plasmid and cultured for several generations. Theplasmids were isolated and digested with NruI, which cleaves in the tetR gene. E. coli (recA-) were transformed with the plasmids and CmRtetS transformants selected. These transformants contained plasmids contg. ***hybrid*** .delta.-endotoxin genes, 10 of which were sequenced.

L3 ANSWER 126 OF 209 CAPLUS COPYRIGHT 1997 ACS
AN 1991:137415 CAPLUS

AN 114:137415
TI Bacillus ***thuringiensis*** strains producing novel endotoxins, the endotoxin genes, and transgenic plants containing the gene
AU Peferoen, Marnix; Lambert, Bart; Joos, Henk
PA Plant Genetic Systems N. V., Belg.
SO Eur. Pat. Appl., 30 pp. CODEN: EPXXDW
PI EP 382990 A1 900822
DS R: AT, BE, CH, DE, ES, FR, GB, GR, IT, LI, LU, NL, SE AI EP 89-400428 890215 DT Patent LA English
AB Two B ***thuringiensis*** strains produce novel endotoxins toxic to Coleoptera. The toxins may be used as insecticides, or the genes may be used to prep. transgenic plants resistant to Coleoptera. The btPGS1208 and btPGS1245 genes were cloned and sequenced. E. coli expression plasmids encoding the complete protoxins, the 66 or 67 kilodalton toxins, or toxin-neo gene product ***fusion*** proteins were constructed. Similar expression vectors for plants were prepd., and Coleoptera-resistant potatoes were produced by std.methods. The LC50 for Colorado potato beetle larvae ingesting toxin-treated leaves was 5-25 .mu.g solubilized crystals/mL.

L3 ANSWER 127 OF 209 CAPLUS COPYRIGHT 1997 ACS
AN 1991:116351 CAPLUS DN 114:116351
TI Novel .delta.-endotoxin gene of Bacillus ***thuringiensis*** kurstaki and expression of ***chimeric*** .delta.-endotoxin genes containing it
AU Ely, Susan; Tippett, Janet Mary
PA Imperial Chemical Industries PLC, UK
SO PCT Int. Appl., 50 pp. CODEN: PIXXD2
PI WO 9003434 A1 900405
DS W: AU, JP, US RW: AT, BE, CH, DE, FR, GB, IT, LU, NL, SE AI WO 89-GB1157 890929 PRAI GB 88-23068 880930 DT Patent LA English
AB The gene for the .delta.-endotoxin of Bacillus ***thuringiensis*** kurstaki A20, that is more active as an insecticide that of the .delta.-endotoxin of B. ***thuringiensis*** kurstaki HD-1, is cloned and expressed as a ***chimeric*** gene with other .delta.-endotoxin sequences in Escherichia coli. The toxicity of .delta.-endotoxin ***fusion*** proteins, prepd. by std. methods, to Plutella xylostella, Heliothis zea, and Trichoplusia ni was studied. At .apprx.500 ppm in the diet the chimeric*** endotoxin was 100 fatal to P. xylostella and caused stunting of 96 of H. zea larvae and of 65 of T. ni larvae.

L3 ANSWER 129 OF 209 CAPLUS COPYRIGHT 1997 ACS
AN 1991:37763 CAPLUS DN 114:37763
TI Larvicidal activity of ***chimeric*** Bacillus ***thuringiensis*** protoxins
AU Raymond, K. C.; John, T. R.; Bulla, L. A., Jr.
CS Dep. Mol. Biol., Univ. Wyoming, Laramie, WY, 82071-3944, USA
SO Mol. Microbiol. (1990), 4(11), 1967-73 CODEN: MOMIEE; ISSN: 0950-382X DT Journal LA English
AB B. ***thuringiensis*** kurstaki (Btk) and subspecies berliner both produce lepidopteran-specific larvicidal protoxins with different activities against the same insect species. Toxic activity resides in the amino-terminal half of both protoxins, whereas the carboxy-terminal half of the mols. is not required for toxicity. The protoxins are 90% homologous, with a major cluster of differences in the amino-terminal half, and a 26 consecutive amino-acid insertion within the carboxy-terminal half of the Btk protoxin. Protoxin ***chimeras*** composed of the amino-terminal half of one subspecies and the carboxy-terminal half of the other were generated. Wild-type and ***chimeric*** protoxins were compared in bioassays against tobacco hornworm larvae. The amino-terminal half, the toxin itself, dictates specific larvicidal activity.

L3 ANSWER 130 OF 209 CAPLUS COPYRIGHT 1997 ACS
AN 1990:606687 CAPLUS DN 113:206687
TI Specificity-determining regions of a lepidopteran-specific insecticidal protein produced by Bacillus ***thuringiensis***
AU Schnepf, H. Ernest; Tomczak, Kathleen; Ortega, Jose Paz; Whiteley, H. R.
CS Dep. Microbiol., Univ. Washington, Seattle, WA, 98195, USA
SO J. Biol. Chem. (1990), 265(34), 20923-30 CODEN: JBCHA3; ISSN: 0021-9258 DT Journal LA English
AB The lepidopteran-specific, insecticidal crystal proteins of B. ***thuringiensis*** vary in toxicity to different species of lepidopteran larvae. Studies are reported of CrylA(a) and CrylA(c), 2 related proteins that have different degrees of toxicity to Heliothis virescens yet very similar degrees of toxicity to Manduca sexta. The amino acid differences between these proteins are located primarily between residues 280 and 722. A series of ***chimeric*** proteins were constructed and their toxicities to both insects detd. The most significant findings arise from the replacement of 3 segments of the crylA(c) gene with homologous portions of the crylA(a) gene: codons 332-428, 429-447, and 448-722. Each of these segments contributed substantially and largely additively toward efficacy for H. virescens. However, replacement of the 429-447 segment of crylA(c) gene with the crylA(a) sequence resulted in a 27-50-fold redn. in toxicity toward M. sexta whereas the redn. in toxicity to H. virescens was only 3-4-fold. Subdivision of the 429-447 segment and replacements involving residues within this segment reduced toxicity to M. sexta by 5- to more than 2000-fold whereas toxicity to H. virescens was only reduced 3-10-fold. These observations indicate that different but overlapping regions of the crylA(c) gene det. specificity to each of the 2 test insects; some of the examd. gene segments interact in detg. specificity, and different sequences in the crylA(a) and crylA(c) genes are required for maximal toxicity to M. sexta.

L3 ANSWER 132 OF 209 CAPLUS COPYRIGHT 1997 ACS
AN 1990:530729 CAPLUS DN 113:130729
TI ***Hybrid*** pesticidal protein toxins, microorganisms producing them, and use of the toxins to control insects
AU Wilcox, Edward; Edwards, David L.; Schwab, George E.; Thompson, Mark; Culver, Paul
PA Mycogen Corp., USA
SO Eur. Pat. Appl., 36 pp. CODEN: EPXXDW
PI EP 340948 A1 891108
DS R: AT, BE, CH, DE, ES, FR, GB, GR, IT, LI, LU, NL, SE AI EP 89-304034 890424 PRAI US 88-187167 880428 DT Patent LA English
AB ***Hybrid*** pesticidal proteins comprising a cytotoxic agent (e.g. ricin or diphtheria toxin) and a pest gut epithelial cell recognition protein, e.g. Bacillus ***thuringiensis*** .delta.-endotoxin, are prepd. with microorganisms. The microorganisms can be used to control insects. A ***chimeric*** gene comprising B. ***thuringiensis*** kurstaki HD-73 .delta.-endotoxin gene fragment ***fused*** to diphtheria toxin B chain DNA was constructed and expressed in Escherichia coli. Novel baculoviruses contg. such genes were constructed; the recombinant Spodoptera exigna nuclear polyhedrosis virus (SeNPV) killed S. exiqua larvae, but not Heliothis zea larvae; a recombinant H. zea nuclear polyhedrosis virus (HzNPV) had the reverse specificity. A ***hybrid*** virus comprising envelope proteins of SeNPV and nucleic acid of HzNPV was prepd. This ***hybrid*** virus killed both types of larvae.

L3 ANSWER 134 OF 209 CAPLUS COPYRIGHT 1997 ACS
AN 1990:493081 CAPLUS DN 113:93081
TI Location of the dipteran specificity region in a lepidopteran-dipteran crystal protein from Bacillus ***thuringiensis***
AU Widner, William R.; Whiteley, H. R.
CS Dep. Microbiol., Univ. Washington, Seattle, WA, 98195, USA
SO J. Bacteriol. (1990), 172(6), 2826-32 CODEN: JOBAAY; ISSN: 0021-9193 DT Journal LA English
AB Two highly related crystal protein genes from B. ***thuringiensis*** subsp. kurstaki HD-1, designated cryIIA and cryIIB (previously named cryB1 and cryB2, resp.), were used to study host range specificity. Their resp. gene products are 87% identical but exhibit different toxicity spectra; CryIIA is toxic to both mosquito and tobacco hornworm larva, whereas CryIIB is toxic only to the latter. Hybrids of the cryIIA and cryIIB genes were generated, and their resultant gene products were assayed for toxicity. A short segment of CryIIA corresponding to residues 307 through 382 was shown to be sufficient for altering host range specificity - i.e., when this region replaced the corresponding segment of CryIIB, the resulting ***hybrid*** protein acquired toxicity against mosquitoes. The CryIIA and CryIIB polypeptides differ by only 18 amino acids in this region, indicating that very few amino acid changes can have a substantial effect on the toxicity spectra of these proteins.

.3 ANSWER 135 OF 209 CAPLUS COPYRIGHT 1997 ACS
AN 1990:492695 CAPLUS DN 113:92695
TI Heterologous expression of a mutated toxin gene from Bacillus ***thuringiensis*** subsp. tenebrionis
AU Rhim, Seong Lyul; Jahn, Norbert; Schnetter, Wolfgang; Geider, Klaus
CS Abt. Mol. Biol., Max-Planck-Inst. Med. Forsch., Heidelberg, D-6900, Fed. Rep. Ger.
SO FEMS Microbiol. Lett. (1990), 66(1-3), 95-9 CODEN: FMLED7; ISSN: 0378-1097 DT Journal LA English
AB Using oligonucleotide probes, a DNA fragment encoding an insecticidal toxin of the coleopteran-specific B. ***thuringiensis*** subsp. tenebrionis was isolated. The gene was altered by site-directed mutagenesis at its 5'-end and adapted for general cloning and expression purposes with a linker including a start codon and new restriction sites. The constructs were inserted into several vector plasmids and expressed in Escherichia coli. Expression in E. coli was strongly enhanced by the lac promoter. A ***fusion*** protein with phage MS2 polymerase was produced together with a 67 kDa protein also found for normal expression of the toxin gene. Synthesis of the latter protein indicated a second ribosome-binding site at the 5'-terminus of the toxin encoding sequence. Toxin-contg. proteins were identified by Western blot anal. The pos. cell exts. from E. coli had insecticidal activity on larvae of the Colorado potato beetle. The cloned gene is not homologous to a previously cloned gene whose gene products were also toxic to coleopteran larvae.

.3 ANSWER 136 OF 209 CAPLUS COPYRIGHT 1997 ACS
AN 1990:435899 CAPLUS DN 113:35899
TI ***Chimeric*** Bacillus ***thuringiensis*** .delta.-endotoxin gene
N Gilroy, Thomas E.
PA Mycogen Corp., USA
SO Eur. Pat. Appl., 11 pp. CODEN: EPXXDW
PI EP 331470 A2 890906
DS R: AT, BE, CH, DE, ES, FR, GB, GR, IT, LI, LU, NL, SE AI EP 89-302049 890301 PRAI US 88-164162 880303 DT Patent LA English
AB A ***chimeric*** .delta.-endotoxin gene contg. sequences from the .delta.-endotoxin genes of Bacillus ***thuringiensis*** ***thuringiensis*** and B. t. kurstaki is constructed and sequences and introduced into Pseudomonas fluorescens. The ***fusion*** protein is potentially active against lepidoptera.

.3 ANSWER 138 OF 209 CAPLUS COPYRIGHT 1997 ACS
AN 1990:401549 CAPLUS DN 113:1549
TI Novel ***hybrid*** Bacillus .delta.-endotoxin for control of Lepidopteran insects
N Gilroy, Thomas E.; Wilcox, Edward R.
PA Mycogen Corp., USA
SO Eur. Pat. Appl., 11 pp. CODEN: EPXXDW
PI EP 325400 A1 890726
DS R: AT, BE, CH, DE, ES, FR, GB, GR, IT, LI, LU, NL, SE AI EP 89-300388 890117 PRAI US 88-146997 880122 DT Patent LA English
AB A novel .delta.-endotoxic gene is constructed from the 5' end of the B ***thuringiensis*** burstaki HO-73 gene and the 3' end of the B. ***thuringiensis*** burstaki HD-1 gene. The ***chimeric*** endotoxin is active against Lepidopteran insects (no data). The gene was used to construct plasmid pM2,16-11 which was used to transform Pseudomonas fluorescens.

.3 ANSWER 140 OF 209 CAPLUS COPYRIGHT 1997 ACS
AN 1990:211954 CAPLUS DN 112:211954
TI Construction of ***chimeric*** insecticidal proteins between the 130-kDa and 135-kDa proteins of Bacillus ***thuringiensis*** subsp. aizawai for analysis of structure-function relationship
AU Nakamura, Keiko; Oshie, Kazuyuki; Shimizu, Masatoshi; Takada, Yoji; Oeda, Kenji; Ohkawa, Hideo
CS Takarazuka Res. Cent., Sumitomo Chem. Co., Ltd., Takarazuka, 665, Japan
SO Agric. Biol. Chem. (1990), 54(3), 715-24 CODEN: ABCHA6; ISSN: 0002-1369 DT Journal LA English
AB Eight ***chimeric*** insecticidal protein (IP) genes were constructed between the 130-kDa and 135-kDa IP genes of B. ***thuringiensis*** subsp. aizawai, and expressed in Escherichia coli JM103 cells. The characterization of the ***chimeric*** IPs indicated that the variable region (VR1) in the amino-terminal half of the IPs is responsible for the insecticidal activity against larvae of Spodoptera litura and Plutella xylostella. The carboxy-terminal half of VR1 was important for the formation of the 60-kDa active fragment in the gut juice of S. litura larvae. Also, combination of the other 2 variable regions (VR2 and VR3), which were in the central and carboxy-terminal portions of the IPs, appeared to be related to the soly. of the IPs in the gut juice.

L3 ANSWER 141 OF 209 CAPLUS COPYRIGHT 1997 ACS
AN 1990:193742 CAPLUS DN 112:193742
TI A translation ***fusion*** product of two different insecticidal crystal protein genes of Bacillus ***thuringiensis*** exhibits an enlarged insecticidal spectrum
AU Honee, Guy; Vriezen, Wim; Visser, Bert
CS Sticht. Ital, Wageningen, 6700 AA, Neth.
SO Appl. Environ. Microbiol. (1990), 56(3), 823-5 CODEN: AEMIDF; ISSN: 0099-2240 DT Journal LA English
AB Two truncated B. ***thuringiensis*** crystal protein genes, belonging to the classes cryIA(b) and cryIC and both coding for insecticidal N-terminal fragments of the corresponding crystal proteins, were translationally ***fused*** . Expression of the gene ***fusion*** in Escherichia coli showed a biol. active protein with a toxicity spectrum that overlapped those of both contributing crystal proteins.

L3 ANSWER 143 OF 209 CAPLUS COPYRIGHT 1997 ACS
AN 1990:173551 CAPLUS DN 112:173551
TI Application of techniques of genetic exchange and genetic engineering to the improvement of the insecticidal properties of Bacillus ***thuringiensis***
AU Bassand, Denis; Jellis, Cindy Lou; Piot, Jean Christophe
CS Sandoz S.A., Basel, Switz.
SO C. R. Acad. Agric. Fr. (1989), 75(6), 127-34 CODEN: CRAFEQ DT Journal LA French
AB Two distinct approaches were selected in order to improve the insecticidal properties of B. ***thuringiensis*** . The first approach, i.e. the use of conjugation methods between strains of various subspecies, resulted in the construction of ***hybrid*** strains exhibiting interesting insecticidal properties. One of the most promising hybrids, L21004, is not only active on lepidopteous larvae, but it also controls some coleopteran species belonging to the Chrysomelidae (Leaf beetles). The second approach, consisting in the use of in vitro chem. mutagenesis and in the cloning of mutants in suitable microorganisms, led to Escherichia coli strains transformed with genetically altered toxin genes. Some of the thus obtained mutants are considerably more active on Heliothis virescens larvae than in the native .delta.-endotoxin.

L3 ANSWER 146 OF 209 CAPLUS COPYRIGHT 1997 ACS
AN 1990:2026 CAPLUS DN 112:2026
TI ***Chimeric*** .delta.-endotoxins of Bacillus ***thuringiensis*** with novel host ranges and their manufacture in Escherichia coli
PA Mycogen Corp., USA
SO Jpn. Kokai Tokkyo Koho, 83 pp. CODEN: JKXXAF
PI JP 62143689 A2 870626 Showa
AI JP 86-295116 861212 PRAI US 85-808129 851212 US 86-904572 860905 DT Patent LA Japanese
AB ***Chimeric*** Bacillus ***thuringiensis*** .delta.-endotoxin proteins with wider host ranges are prepd. by recombining in vitro the coding sequences for the variable regions (k-1 and k-73 regions) of the .delta.-endotoxins of B. ***thuringiensis*** kurstaki HD-1 and B. ***thuringiensis*** kurstaki HD 73. Plasmid pEW3 contg. the gene encoding k-1 and k-73 regions was constructed and expressed in Escherichia coli. The LD50 of ***chimeric*** toxin EW3 (k-1/k-73) to Tricholplasia ni and Spodoptera exiqua was 4.3 and 12.3 O.D.575/mL.

L3 ANSWER 148 OF 209 CAPLUS COPYRIGHT 1997 ACS
AN 1989:569356 CAPLUS DN 111:169356
TI Insecticidal activity of a peptide containing the 30th to 695th amino acid residues of the 130-kDa protein of Bacillus ***thuringiensis*** var. israelensis
AU Yoshida, Kenichi; Matsushima, Yutaka; Sen, Kikuo; Sakai, Hiroshi; Komano, Tohru
CS Dep. Agric. Chem., Kyoto Univ., Kyoto, 606, Japan
SO Agric. Biol. Chem. (1989), 53(8), 2121-7 CODEN: ABCHA6; ISSN: 0002-1369 DT Journal LA English
AB B. ***thuringiensis*** var. israelensis produces 130-kDa proteins which are toxic to mosquito larvae. The ISRH4 gene encoding 1180 amino acids of the 130-kDa insecticidal protein was ***fused*** with lacZ' on a plasmid, pUC19, and sequentially deleted from the C-terminus to construct a series of deletion mutants. All the deletion mutant genes directed the prodn. of truncated ISRH4 proteins ***fused*** with the .alpha.-complementing fragment of .beta.-galactosidase in Escherichia coli cells in the presence of iso-Pr .beta.-D-thiogalactopyranoside. Anal. of the mosquito larvicidal activity of deletion mutant proteins revealed that the N-terminal 29 amino acids and the C-terminal 485 amino acids could be removed without loss of the activity.

L3 ANSWER 155 OF 209 CAPLUS COPYRIGHT 1997 ACS
AN 1989:452127 CAPLUS DN 111:52127
TI Location of the Bombyx mori specificity domain on a Bacillus ***thuringiensis*** .delta.-endotoxin protein
AU Ge, Albert Z.; Shivarova, Nedka I.; Dean, Donald H.
CS Dep. Biochem., Ohio State Univ., Columbus, OH, 43210, USA
SO Proc. Natl. Acad. Sci. U. S. A. (1989), 86(11), 4037-41 CODEN: PNASA6; ISSN: 0027-8424 DT Journal LA English
AB B. ***thuringiensis*** produces different types of insecticidal crystal proteins (ICPs) or .delta.-endotoxins. In an effort to identify the insect specificity of ICP toxins, two icp genes were cloned into the Escherichia coli expression vector pKK223-3, and bioassays were performed with purified crystals. The type A protein [from an icpA1, or 4.5-kilobase (kb) gene, from B. ***thuringiensis*** var kurstaki HD-1] was 400 times more active against B. mori type C protein (from an icpC73, or 6.6-kb gene, from B. ***thuringiensis*** var kurstaki HD-244). The type C protein was 9 times more active against Trichoplusia ni than the type Arotein, while both have similar activity against Manduca sexta. To locate the specificity domain of the type A protein for B. mori, site-directed mutagenesis was used to introduce or remove restriction enzyme sites, facilitating the exchange of regions of the two genes. The ***hybrid*** genes were overexpressed, and purified ICP was used in bioassays. The B. mori specificity domain for the ICP A toxin is located in the amino-terminal portion of the hypervariable region between amino acids 332 and 450.

L3 ANSWER 157 OF 209 CAPLUS COPYRIGHT 1997 ACS
AN 1989:418805 CAPLUS DN 111:18805
TI ***Chimeric*** pesticide proteins of Bacillus ***thuringiensis*** and their recombinant manufacture
IN Nakamura, Keiko; Oita, Kenji; Oshiye, Kazuyuki; Shimizu, Masatoshi; Takada, Yasushi; Nakayama, Isamu; Okawa, Hideo
PA Sumitomo Chemical Co., Ltd., Japan
SO Jpn. Kokai Tokkyo Koho, 22 pp. CODEN: JKXXAF
PI JP 63137684 A2 880609 Showa
AI JP 86-283228 861127 DT Patent LA Japanese
AB The genes encoding pesticide proteins of 125 kd and 130 kd of B. ***thuringiensis*** are used to construct recombinant DNA encoding the ***chimeric*** pesticide proteins. The DNA encoding 125 kd protein and 130 kd protein were isolated form plasmids pTB1 and pKC6, resp. The restriction enzyme fragments KpnI-PstI (a1), KpnI-HindIII (a2), and HindIII-PstI (a3) of 125 kd protein gene as well as the counterpart fragments (C1, C2, and C3) of 130 kd protein gene were used to construct 6 expression plasmids contg. 6 variable combinations such as a1a2c3, a1c2a3, etc. The ***chimeric*** genes were expressed in transformed Escherichia coli. The pesticidal effect of the ***chimeric*** proteins were demonstrated.

L3 ANSWER 160 OF 209 CAPLUS COPYRIGHT 1997 ACS
AN 1989:189316 CAPLUS DN 110:189316
TI Novel Bacillus ***thuringiensis*** with altered insecticidal activities prepared by protoplast ***fusion***
IN Krieg, Wolfgang; Zaehner, Hans; Bernhard, Konrad; Schall, Dietmar
PA BASF A.-G., Fed. Rep. Ger.
SO Eur. Pat. Appl., 12 pp. CODEN: EPXXDW
PI EP 288829 A1 881102
DS R: AT, BE, CH, DE, FR, GB, IT, LI, NL AI EP 88-105964 880414 PRAI DE 87-3713946 870425 DT Patent LA German
AB B. thuringgiensis strains prepd. by protoplast ***fusion*** of strains producing different endotoxins have altered insecticidal activities relative to either parent. B. ***thuringiensis*** DSM4082 was created by ***fusion*** of a strain of pathotype A (active against Lepidoptera) with a strain of pathotype C (active against Coleoptera). The novel strain had a higher activity against larvae of destructive moths and beetles, e.g. Plutella maculipennis, Spodoptera littoralis, and Leptinotarsa decemlineata.

L3 ANSWER 163 OF 209 CAPLUS COPYRIGHT 1997 ACS
AN 1988:544579 CAPLUS DN 109:144579
TI Obtaining a ***hybrid*** for a new insecticide by means of protoplast ***fusion***
AU Wang, Yuewu; Chen, Yuehua; Chen, Ning
CS Biol. Dep., Nankai Univ., Tianjin, Peop. Rep. China
SO Kexue Tongbao (Foreign Lang. Ed.) (1988), 33(11), 963 CODEN: KHTPBU; ISSN: 0454-0948 DT Journal LA English
AB To obtain a new ***hybrid*** the protoplast ***fusion*** technique was used with 2 strains of bacteria, Bacillus sphaericus Ts-1 which has Str resistance and high toxicity to Culex mosquitoes and wild type B. ***thuringiensis*** H4 which is Amp resistance and toxic to Ostrinia nubilalis. Several ***fusion*** hybrids, F-e, F-f, and F-9, were obtained, and these hybrids were toxic to wigglers and worms. After 22 generations, they always keep the original characteristics. Because of the use of DNase in the expt., it was not possible for the hybrids to have come from the transformation. The efficiencies of the hybrids F-e and F-9 to kill mosquitoes and O. nubilalis were >90 and 80%, resp. The efficiencies of F-f to kill mosquitoes and O. nubilalis were >90 and 60-70%, resp. These results indicate that these hybrids contain 2 kinds of toxic proteins so that they can kill both Lepidoptera larva and Diptera (wigglers). Serol. tests indicate that F-e, F-9, F-f and Ts-1 have the same H antigen, but H4 does not.

L3 ANSWER 164 OF 209 CAPLUS COPYRIGHT 1997 ACS
AN 1988:543900 CAPLUS DN 109:143900
TI Engineering of insect resistant plants using a B. ***thuringiensis*** gene
AU Vaeck, M.; Hoefte, H.; Reynaerts, A.; Leemans, J.; Van Montagu, M.; Zabeau, M.
CS Plant Genet. Syst. N. V., Ghent, Belg.
SO UCLA Symp. Mol. Cell. Biol., New Ser. (1987), 48(Mol. Strategies Crop Prot.), 355-66 CODEN: USMBD6; ISSN: 0735-9543 DT Journal LA English
AB A crystal protein gene (bt2) has been cloned from plasmid DNA of B. ***thuringiensis*** (B.t.) berliner 1715 and directs the synthesis of a 130 kd protein (Bt2) in E. coli which is toxic to larvae of Pieris brassicae and Manduca sexta. Treatment of the Bt2 protein with trypsin or chymotrypsin yields a 60 kd protease resistant fragment which is fully toxic towards insect larvae in vivo and insect cell lines in vitro. The minimal portion of the Bt2 protein required for toxicity has been mapped by deletion anal. and coincides with the 60 kd protease resistant Bt2-fragment. Tobacco plant cells have been transformed with ***chimeric*** toxin genes using a Ti plasmid vector. Transformed plants express afunctional toxin and exhibit resistance against insect larvae.

L3 ANSWER 172 OF 209 CAPLUS COPYRIGHT 1997 ACS
AN 1988:126648 CAPLUS DN 108:126648
TI ***Fusion*** proteins with both insecticidal and neomycin phosphotransferase II activity
AU Hoefte, Herman; Buyssens, Saskia; Vaeck, Mark; Leemans, Jan
CS Plant Genet. Syst. N. V. J., Ghent, 9000, Belg.
SO FEBS Lett. (1988), 226(2), 364-70 CODEN: FEBLAL; ISSN: 0014-5793 DT Journal LA English

AB ***Hybrid*** proteins consisting of N-terminal fragments of increasing length of a Bacillus ***thuringiensis*** insecticidal protein (Bt2) ***fused*** to neomycin phosphotrasferase II NPTII) were produced in Escherichia coli. The min. fragment required for insect toxicity is comprised of the first 607 amino acids of Bt2. ***Fusion*** proteins not contg. this min. fragment were non-toxic. The NPTII activity of the different non-toxic ***hybrid*** proteins varied considerably but was not correlated with the length of the Bt2 fragment. ***Fusion*** proteins including the min. toxic fragment of Bt2 exhibited insecticidal and d NPTII activity comparable to that of the individual proteins. This was largely independent of the ***fusion*** point within Bt2. Apparently, the conformation of the Bt2 polypeptide exerts an important influence on the enzymic activity of the ***fused*** NPTII protein. The combination of insecticidal activity and a dominant selectable trait into one protein offers important advantages for the generation of insect resistant transgenic plants.

L3 ANSWER 189 OF 209 CAPLUS COPYRIGHT 1997 ACS
AN 1985:417768 CAPLUS DN 103:17768
TI Delineation of a toxin-encoding segment of a Bacillus ***thuringiensis*** crystal protein gene
AU Schnepf, H. Ernest; Whiteley, H. R.
CS Dep. Microbiol. Immunol., Univ. Washington, Seattle, WA, 98195, USA
SO J. Biol. Chem. (1985), 260(10), 6273-80 CODEN: JBCHA3; ISSN: 0021-9258 DT Journal LA English
AB Crystals of B. ***thuringiensis*** kurstaki HD-1-Dipel contain a 134,000-mol.-wt. protoxin which can be cleaved by proteolysis to a peptide of .apprx.70,000 mol. wt.; this peptide is lethal to lepidopteran larvae. The peptides produced by recombinant Escherichia coli strains bearing deletions and ***fusions*** of the protoxin gene were analyzed in order to delineate the portion of the gene which encodes the toxic peptide. The recombinant strains produced the toxic peptide as well as larger peptides whose size was related to the length of the deleted gene. The results indicate that the amino-terminal 55% of the protoxin protein is sufficient for toxicity. Whereas 2 different gene **fusions*** to the 10th codon allowed the synthesis of toxic polypeptides, **fusions*** to the 50th codon did not. Some 3' end deletions up to the 645th codon allowed synthesis of the toxic peptide, whereas a deletion to the 603rd codon yielded a nontoxic peptide. Some of the 5'- and 3'-end alterations to the gene caused changes in the proteolytic cleavage patterns of the polypeptides synthesized by E. coli, suggesting that the alterations led to conformational changes in the proteins. The presence of different 3'-end segments affected the levels of synthesis of the altered crystal proteins.

L7 ANSWER 1 OF 72 CAPLUS COPYRIGHT 1997 ACS
TI Cross-resistance of the diamondback moth indicates altered interactions with domain II of Bacillus ***thuringiensis*** toxins

L7 ANSWER 2 OF 72 CAPLUS COPYRIGHT 1997 ACS
TI Recombinant cyanobacteria producing CryIVD ***endotoxin*** and its use as biopesticide against Diptera

L7 ANSWER 3 OF 72 CAPLUS COPYRIGHT 1997 ACS
TI Lepidopteran pesticidal compositions comprising ***chimeric*** CryIF and CryIA(c) .delta.-endotoxins

L7 ANSWER 4 OF 72 CAPLUS COPYRIGHT 1997 ACS
TI Domain III substitution in Bacillus ***thuringiensis*** delta- ***endotoxin*** CryIA(b) results in superior toxicity for Spodoptera exigua and altered membrane protein recognition

L7 ANSWER 5 OF 72 CAPLUS COPYRIGHT 1997 ACS
TI Antibodies which bind to insect gut proteins and their use in preparation of immunotoxins

L7 ANSWER 6 OF 72 CAPLUS COPYRIGHT 1997 ACS
TI Recombinant preparation of ***chimeric*** Bacillus ***thuringiensis*** .delta.- ***endotoxin*** of cryIC and cryIA(b) with improved toxicity

L7 ANSWER 7 OF 72 CAPLUS COPYRIGHT 1997 ACS
TI ***Chimeric*** Bacillus ***thuringiensis*** .delta.- ***endotoxin*** expression in Pseudomonas fluorescens and its improvement

L7 ANSWER 8 OF 72 CAPLUS COPYRIGHT 1997 ACS
TI Development of insect resistance in tomato plants expressing the .delta.- ***endotoxin*** gene of Bacillus ***thuringiensis*** subsp. tenebrionis

L7 ANSWER 9 OF 72 CAPLUS COPYRIGHT 1997 ACS
TI Domain III exchanges of Bacillus ***thuringiensis*** cryIa toxins affect binding to different gypsy moth midgut receptors

L7 ANSWER 10 OF 72 CAPLUS COPYRIGHT 1997 ACS
TI ***Hybrid*** toxins of Bacillus ***thuringiensis***

L7 ANSWER 11 OF 72 CAPLUS COPYRIGHT 1997 ACS
TI Insecticidal proteins constructed from Bacillus ***thuringiensis*** .delta.- ***endotoxin*** and Androctonus australis neurotoxin AaHIT

L7 ANSWER 12 OF 72 CAPLUS COPYRIGHT 1997 ACS
TI Transgenic tobacco plants with efficient insect resistance

L7 ANSWER 13 OF 72 CAPLUS COPYRIGHT 1997 ACS
TI The effect of ***toxin*** -producing Rhizobium strains, on larvae of Sitona flavescens feeding on legume roots and nodules. [Erratum to document cited in CA121:274435]

L7 ANSWER 14 OF 72 CAPLUS COPYRIGHT 1997 ACS
TI Insecticidal ***fusion*** proteins of Bacillus ***thuringiensis*** var. kurstaki HD-1

L7 ANSWER 15 OF 72 CAPLUS COPYRIGHT 1997 ACS
TI Insect resistance of transgenic plants that express modified Bacillus ***thuringiensis*** cryIA(b) and cryIC genes: a resistance management strategy

L7 ANSWER 16 OF 72 CAPLUS COPYRIGHT 1997 ACS
TI Protoplast ***fusion*** of Bacillus subtilis and Bacillus ***thuringiensis*** for breeding of pesticidal strains against plant pathogens

L7 ANSWER 17 OF 72 CAPLUS COPYRIGHT 1997 ACS
TI The effect of ***toxin*** -producing Rhizobium strains, on larvae of Sitona flavescens feeding on legume roots and nodules

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L7 ANSWER 18 OF 72 CAPLUS COPYRIGHT 1997 ACS
TI Expression of the insecticidal ***crystal*** ***protein*** gene from a Gram-positive Bacillus ***thuringiensis*** in a Gram-negative Pseudomonas fluorescens mediated by protoplast ***fusion***

L7 ANSWER 19 OF 72 CAPLUS COPYRIGHT 1997 ACS
TI Intracellular proteolysis and limited diversity of the Bacillus ***thuringiensis*** CryIA family of the insecticidal crystal proteins

L7 ANSWER 20 OF 72 CAPLUS COPYRIGHT 1997 ACS
TI Use of an operon ***fusion*** to induce expression and crystallization of a Bacillus ***thuringiensis*** .delta.- ***endotoxin*** encoded by a cryptic gene

L7 ANSWER 21 OF 72 CAPLUS COPYRIGHT 1997 ACS
TI Primary structure of cryX, the novel .delta.- ***endotoxin*** -related gene from Bacillus ***thuringiensis*** spp. galleriae

L7 ANSWER 22 OF 72 CAPLUS COPYRIGHT 1997 ACS
TI Construction of a gene for a ***hybrid*** protein based on Bacillus ***thuringiensis*** .delta.- ***endotoxin*** CryIA(a) and CryIIIA sequences and expression of its derivatives in Escherichia coli

L7 ANSWER 23 OF 72 CAPLUS COPYRIGHT 1997 ACS
TI Expression of ***endotoxin*** gene from Bacillus ***thuringiensis*** with insect bacutovirus transfer vector in Escherichia coli

L7 ANSWER 24 OF 72 CAPLUS COPYRIGHT 1997 ACS
TI Transformation of Liquidambar styraciflua using Agrobacterium tumefaciens

L7 ANSWER 25 OF 72 CAPLUS COPYRIGHT 1997 ACS
TI Simple method to evaluate sterilization of recombinant Pseudomonas carrying insecticidal protein gene

L7 ANSWER 26 OF 72 CAPLUS COPYRIGHT 1997 ACS

I Synthetic genes for delta-endotoxins optimized for expression in maize

7 ANSWER 27 OF 72 CAPLUS COPYRIGHT 1997 ACS
I Expression of a ***hybrid*** gene for bifunctional insect ***toxin*** -glucuronidase protein in transgenic tobacco

7 ANSWER 28 OF 72 CAPLUS COPYRIGHT 1997 ACS
I Expression of a ***chimeric*** CaMV 35S Bacillus ***thuringiensis*** insecticidal protein gene in transgenic tobacco. [Erratum to document cited in CA118(3):17151c]

7 ANSWER 29 OF 72 CAPLUS COPYRIGHT 1997 ACS
I Suppression of protein structure destabilizing mutations in Bacillus ***thuringiensis*** .delta.-endotoxins by second site mutations

7 ANSWER 30 OF 72 CAPLUS COPYRIGHT 1997 ACS
I Transgenic tomato plants expressing insecticidal activity against coleopteran larvae

7 ANSWER 31 OF 72 CAPLUS COPYRIGHT 1997 ACS
I Expression of a ***chimeric*** CaMV 35S Bacillus ***thuringiensis*** insecticidal protein gene in transgenic tobacco

7 ANSWER 32 OF 72 CAPLUS COPYRIGHT 1997 ACS
I Transgenic rice plant of a superior Chinese cultivar Zhonghua No. 11 containing the B. t. .delta.- ***endotoxin*** gene in its genome

7 ANSWER 33 OF 72 CAPLUS COPYRIGHT 1997 ACS
I Extending the host range of insecticidal proteins using peptides that bind gut cells

7 ANSWER 34 OF 72 CAPLUS COPYRIGHT 1997 ACS
I Strains of Bacillus ***thuringiensis*** and their genes encoding insecticidal toxins

7 ANSWER 35 OF 72 CAPLUS COPYRIGHT 1997 ACS
I Construction of genes for bifunctional derivatives of Bacillus ***thuringiensis*** var. kurstaki insect ***toxin*** for expression in transgenic plants

7 ANSWER 36 OF 72 CAPLUS COPYRIGHT 1997 ACS
I Isolation and cloning of Bacillus ***thuringiensis*** var Kurstaki HD73 ***toxin*** gene and construction of a ***chimeric*** gene for expression in plants.

7 ANSWER 37 OF 72 CAPLUS COPYRIGHT 1997 ACS
I A temperature-stable Bacillus ***thuringiensis*** .delta.- ***endotoxin*** analog

7 ANSWER 38 OF 72 CAPLUS COPYRIGHT 1997 ACS
I Development of insect resistant plants

7 ANSWER 39 OF 72 CAPLUS COPYRIGHT 1997 ACS
I Generation of functional Bacillus ***thuringiensis*** ***toxin*** ***hybrid*** genes by in vivo recombination

7 ANSWER 40 OF 72 CAPLUS COPYRIGHT 1997 ACS
I In vivo generation of hybrids between two Bacillus ***thuringiensis*** insect- ***toxin*** -encoding genes

7 ANSWER 41 OF 72 CAPLUS COPYRIGHT 1997 ACS
I Functional domains of Bacillus ***thuringiensis*** insecticidal crystal proteins. Refinement of Heliothis virescens and Trichoplusia ni specificity domains on CryIA(c)

7 ANSWER 42 OF 72 CAPLUS COPYRIGHT 1997 ACS
I Insecticidal activity of Bacillus ***thuringiensis*** ***chimeric*** protoxins

7 ANSWER 43 OF 72 CAPLUS COPYRIGHT 1997 ACS
I Activation of a cryptic ***crystal*** ***protein*** gene of Bacillus ***thuringiensis*** subspecies kurstaki by gene ***fusion*** and determination of the *crystal*** ***protein*** insecticidal specificity

7 ANSWER 44 OF 72 CAPLUS COPYRIGHT 1997 ACS
I New functional Bacillus ***thuringiensis*** .delta.- ***endotoxin*** ***hybrid*** genes obtained by in vivo recombination

7 ANSWER 45 OF 72 CAPLUS COPYRIGHT 1997 ACS
I Transgenic plants for the prevention of development of insects resistant to Bacillus ***thuringiensis*** toxins

7 ANSWER 46 OF 72 CAPLUS COPYRIGHT 1997 ACS
I Bacillus ***thuringiensis*** strains producing novel endotoxins, the ***endotoxin*** genes, and transgenic plants containing the gene

7 ANSWER 47 OF 72 CAPLUS COPYRIGHT 1997 ACS
I Novel .delta.- ***endotoxin*** gene of Bacillus ***thuringiensis*** kurstaki and expression of ***chimeric*** .delta.- ***endotoxin*** genes containing it

7 ANSWER 48 OF 72 CAPLUS COPYRIGHT 1997 ACS
I Cloning of Bacillus ***thuringiensis*** bt4 and bt18 genes, and lepidoptera-resistant plants containing these genes

7 ANSWER 49 OF 72 CAPLUS COPYRIGHT 1997 ACS
I ***Hybrid*** pesticidal protein toxins, microorganisms producing them, and use of the toxins to control insects

7 ANSWER 50 OF 72 CAPLUS COPYRIGHT 1997 ACS
I Differential expression of the 3 .delta.- ***endotoxin*** genes in Bacillus ***thuringiensis*** subsp. kurstaki HD1

7 ANSWER 51 OF 72 CAPLUS COPYRIGHT 1997 ACS
I ***Chimeric*** Bacillus ***thuringiensis*** .delta.- ***endotoxin*** gene

7 ANSWER 52 OF 72 CAPLUS COPYRIGHT 1997 ACS
I Novel ***hybrid*** Bacillus .delta.- ***endotoxin*** for control of Lepidopteran insects

7 ANSWER 53 OF 72 CAPLUS COPYRIGHT 1997 ACS
I Transgenic rice plants produced by direct uptake of .delta.- ***endotoxin*** protein gene from Bacillus ***thuringiensis*** into rice protoplasts

7 ANSWER 54 OF 72 CAPLUS COPYRIGHT 1997 ACS
I A translation ***fusion*** product of two different insecticidal ***crystal*** ***protein*** genes of Bacillus ***thuringiensis*** exhibits an enlarged insecticidal spectrum

7 ANSWER 55 OF 72 CAPLUS COPYRIGHT 1997 ACS
I Cloning and expression in microorganisms of ***endotoxin*** gene of Bacillus ***thuringiensis*** tenebrionis

7 ANSWER 56 OF 72 CAPLUS COPYRIGHT 1997 ACS
I ***Chimeric*** .delta.-endotoxins of Bacillus ***thuringiensis*** with novel host ranges and their manufacture in Escherichia coli

7 ANSWER 57 OF 72 CAPLUS COPYRIGHT 1997 ACS
I Cloning and expression of genes encoding proteins with larvicidal activity against Lepidoptera

7 ANSWER 58 OF 72 CAPLUS COPYRIGHT 1997 ACS
I Accumulation of the insecticidal ***crystal*** ***protein*** of Bacillus ***thuringiensis*** subsp. kurstaki in post-exponential-phase Bacillus subtilis

7 ANSWER 59 OF 72 CAPLUS COPYRIGHT 1997 ACS
I Novel .delta.- ***endotoxin*** gene from Bacillus ***thuringiensis*** israelensis and its expression and use as insecticide

7 ANSWER 60 OF 72 CAPLUS COPYRIGHT 1997 ACS
I Regeneration of Zea mays protoplasts containing a cloned Bacillus ***thuringiensis*** ***crystal*** ***protein*** gene

L7 ANSWER 61 OF 72 CAPLUS COPYRIGHT 1997 ACS
TI Novel Bacillus ***thuringiensis*** with altered insecticidal activities prepared by protoplast ***fusion***

L7 ANSWER 62 OF 72 CAPLUS COPYRIGHT 1997 ACS
TI Expression of Bacillus ***endotoxin*** gene in cyanobacteria, and use of the transformants as an insecticide

L7 ANSWER 63 OF 72 CAPLUS COPYRIGHT 1997 ACS
TI Engineering of insect resistant plants using a B. ***thuringiensis*** gene

L7 ANSWER 64 OF 72 CAPLUS COPYRIGHT 1997 ACS
TI Application of genetic engineering technology in the creation of tobaccos resistant to insects

L7 ANSWER 65 OF 72 CAPLUS COPYRIGHT 1997 ACS
TI Insect resistance in transgenic plants expressing *Bacillus* ***thuringiensis*** ***toxin*** genes

L7 ANSWER 66 OF 72 CAPLUS COPYRIGHT 1997 ACS
TI ***Fusion*** proteins with both insecticidal and neomycin phosphotransferase II activity

L7 ANSWER 67 OF 72 CAPLUS COPYRIGHT 1997 ACS
TI Bacillus ***thuringiensis*** delta.- ***endotoxin*** expressed in transgenic Nicotiana tabacum provides resistance to Lepidopteran insects

L7 ANSWER 68 OF 72 CAPLUS COPYRIGHT 1997 ACS
TI Expression of a cloned Bacillus ***thuringiensis*** ***crystal*** ***protein*** gene in Escherichia coli

L7 ANSWER 69 OF 72 CAPLUS COPYRIGHT 1997 ACS
TI Insecticidal delta.- ***endotoxin*** production by genetically engineered Escherichia coli

L7 ANSWER 70 OF 72 CAPLUS COPYRIGHT 1997 ACS
TI ***Hybrid*** Bacillus ***thuringiensis*** producing .delta.-endotoxins of kurstaki and tenebrionis strains

L7 ANSWER 71 OF 72 CAPLUS COPYRIGHT 1997 ACS
TI New strains of Bacillus ***thuringiensis*** produced by protoplast ***fusion***

L7 ANSWER 72 OF 72 CAPLUS COPYRIGHT 1997 ACS
TI Modifying plants by genetic engineering to combat or control insects

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FILE 'USPAT' ENTERED AT 09:27:49 ON 13 MAY 1997)

.1 772 S THURINGIENSIS
.2 5276 S ENDOTOXIN OR TOXIN OR CRYSTAL PROTEIN OR CRYI?
.3 64636 S FUSION OR CHIMER? OR HYBRID
.4 160 S L3(5N)L2
.5 48 S L1 AND L4
.6 21 S L1(P)L4
.7 27 S L5 NOT L6

.6

. 5,595,733, Jan. 21, 1997, Methods for protecting ZEA mays plants against pest damage; Gleta Carswell, et al., 424/93.21; 536/23.71; 800/205 [IMAGE AVAILABLE]

!. 5,593,881, Jan. 14, 1997, Bacillus thuringiensis delta-endotoxin; Mark Thompson, et al., 435/418, 252.3, 320.1; 536/23.71 [IMAGE AVAILABLE]

). 5,583,036, Dec. 10, 1996, Regeneration of cotton plant in suspension culture; Thirumale S. Rangan, et al., 435/427 [IMAGE AVAILABLE]

). 5,545,565, Aug. 13, 1996, Transformation vectors allowing expression of foreign polypeptide endoxins from Bacillus thuringiensis in plants; Henri M. J. De Greve, et al., 435/320.1, 69.1, 172.3; 514/12 [IMAGE AVAILABLE]

). 5,527,883, Jun. 18, 1996, Delta-endotoxin expression in pseudomonas fluorescens; Mark Thompson, et al., 530/350; 435/252.34, 320.1; 536/23.4, 23.71; 935/10, 29, 72 [IMAGE AVAILABLE]

). 5,518,897, May 21, 1996, Recombinant biopesticide and method of use thereof; S. Edward Stevens, Jr., et al., 435/69.1; 424/93.1, 93.2, 93.4, 93.461; 435/252.3, 252.5, 320.1, 832; 536/22.1, 23.1, 23.4, 23.7, 23.71 [IMAGE AVAILABLE]

'. 5,508,264, Apr. 16, 1996, Pesticidal compositions; Gregory A. Bradfisch, et al., 514/12; 530/350 [IMAGE AVAILABLE]

). 5,495,071, Feb. 27, 1996, Insect resistant tomato and potato plants; David A. Fischhoff, et al., 800/205; 435/69.1, 172.3, 320.1, 411, 417, 418; 514/12; 536/23.71; 800/DIG.42, DIG.44 [IMAGE AVAILABLE]

). 5,424,409, Jun. 13, 1995, DNA constructs encoding Bacillus thuringiensis toxins from strain A20; Susan Ely, et al., 536/23.71; 424/93.461; 536/23.4 [IMAGE AVAILABLE]

10. 5,422,120, Jun. 6, 1995, Heterovesicular liposomes; Sinil Kim, 424/450; 264/4.1, 4.3, 4.6; 436/829 [IMAGE AVAILABLE]

11. 5,350,689, Sep. 27, 1994, Zea mays plants and transgenic Zea mays plants regenerated from protoplasts or protoplast-derived cells; Ray Shillito, et al., 435/412, 421 [IMAGE AVAILABLE]

12. 5,317,096, May 31, 1994, Transformation vectors allowing expression of foreign polypeptide endotoxins from Bacillus thuringiensis in plants; Henri M. J. De Greve, et al., 536/23.71 [IMAGE AVAILABLE]

13. 5,306,628, Apr. 26, 1994, Method and means for extending the host range of insecticidal proteins; Natarajan Sivasubramanian, et al., 435/69.7, 320.1; 530/350; 536/23.71; 935/47 [IMAGE AVAILABLE]

14. 5,290,914, Mar. 1, 1994, Hybrid diphtheria-B.t. pesticidal toxins; Edward Wilcox, et al., 530/350; 435/69.7; 514/2, 12; 935/47 [IMAGE AVAILABLE]

15. 5,254,799, Oct. 19, 1993, Transformation vectors allowing expression of Bacillus thuringiensis endotoxins in plants; Henri M. J. De Greve, et al., 800/205; 435/418; 800/250, DIG.9; 935/67 IMAGE AVAILABLE]

16. 5,244,802, Sep. 14, 1993, Regeneration of cotton; Thirumale S. Rangan, 435/427; 47/58 [IMAGE AVAILABLE]

17. 5,177,308, Jan. 5, 1993, Insecticidal toxins in plants; Kenneth A. Barton, et al., 800/205; 435/172.3, 320.1; 800/DIG.9, DIG.43; 935/67 [IMAGE AVAILABLE]

18. 5,143,905, Sep. 1, 1992, Method and means for extending the host range of insecticidal proteins; Natarajan Sivasubramanian, et al., 514/21; 424/405; 435/69.7; 514/8, 12; 530/350, 409 IMAGE AVAILABLE]

19. 5,128,130, Jul. 7, 1992, Hybrid Bacillus thuringiensis gene, plasmid and transformed Pseudomonas fluorescens; Thomas E. Gilroy, et al., 424/93.2; 435/69.1, 71.2, 91.41, 170, 172.1, 172.3, 252.3, 320.1, 832, 848, 874; 530/350; 536/23.71; 935/6, 9, 10, 22, 27, 59, 60, 61 [IMAGE AVAILABLE]

20. 5,071,654, Dec. 10, 1991, Ion channel properties of delta endotoxins; Leigh H. English, 424/405, 93.461, 450; 435/29, 252.31; 530/324, 825 [IMAGE AVAILABLE]

21. 5,055,294, Oct. 8, 1991, **Chimeric** Bacillus **thuringiensis** **crystal** **protein** gene comprising HD-73 and Berliner 1715 toxin genes, transformed and expressed in Pseudomonas fluorescens; Thomas E. Gilroy, 424/93.2, 93.21; 435/69.1, 69.7, 172.3, 252.3, 252.31, 252.32, 252.33, 252.34, 254.11, 254.2, 320.1; 536/23.71; 935/64, 72 [IMAGE AVAILABLE]

US PAT NO: 5,595,733 [IMAGE AVAILABLE] L6: 1 of 21
DETDESC:DETD(377)
Construction of pTOX, containing a **chimeric** gene encoding the insecticidal **toxin** gene of Bacillus **thuringiensis** var tenebrionis

DETD(380)
Construction of pSAN, containing a **chimeric** gene encoding the insecticidal **toxin** gene of Bacillus **thuringiensis** strain san diego

US PAT NO: 5,593,881 [IMAGE AVAILABLE] L6: 2 of 21
ABSTRACT:
An improved Bacillus **thuringiensis** (B.t.) delta-endotoxin is created by the modification of the gene encoding the toxin. The toxicity of aB.t. toxin was improved by replacing the native protoxin segment with an alternate protoxin segment by constructing a **chimeric** **toxin** gene.

SUMMARY:BSUM(6)
The . . . Natl. Acad. Sci. U.S.A. 78:2893-2897). U.S. Pat. No. 4,448,885 and U.S. Pat. No. 4,467,036 both disclose the expression of B.t. **crystal** **protein** in E. coli. **Hybrid** B.t. crystal proteins have been constructed that exhibit increased toxicity and display an expanded host range to a target pest. See U.S. Pat. Nos. 5,238,130 and 5,055,294. U.S. Pat. Nos. 4,797,276 and 4,853,331 disclose B. **thuringiensis** strain tenebrionis (a.k.a. M-7, a.k.a.B.t. san diego) which can be used to control coleopteran pests in various environments. .S. Pat.. . . Pat. No. 4,849,217 discloses B.t. isolates which have activity against the alfalfa weevil. U.S. Pat. No. 5,208,077 discloses coleopteran-active Bacillus **thuringiensis** isolates. U.S. Pat. No. 5,151,363 and U.S. Pat. No. 4,948,734 disclose certain isolates of B.t. which have activity against nematodes.. . .

BSUM(11)
The subject invention concerns the discovery that the activity of a Bacillus **thuringiensis** (B.t.) .delta.-endotoxin can be substantially improved by replacing native protoxin amino acids with an alternate protoxin sequence, yielding a **chimeric** **toxin**. In a specific embodiment of the subject invention, a **chimeric** **toxin** is assembled by substituting all or part of the cryIA(b) protoxin segment for all or part of the native cryIC protoxin segment. The cryIC/cryIA(b) **chimeric** **toxin** demonstrates an increased toxicity over the cryIC/cryIC toxin produced by the native gene.

DETDESC:DETD(2)
The subject invention concerns the discovery of highly active chimeric Bacillus **thuringiensis** toxins. These chimeric toxins are created by replacing all or part of the native protoxin segment of a full length B.t. toxin with an alternate protoxin segment. In a preferred embodiment, the **chimeric** **toxin** comprises a cryIA(b) C-terminal protoxin portion and a cryIC core N-terminal toxin portion. As used herein, reference to a "core". . .

We claim:
1. An isolated DNA molecule comprising a nucleotide sequence encoding a **chimeric** Bacillus **thuringiensis** **toxin** of approximately 1150 to 1200 amino acids, wherein said toxin comprises a cryIC core N-terminal toxin portion having a sequence. . . amino acids, wherein the amino acid sequence from the end of said core N-terminal sequence to the C-terminus of the **chimeric** **toxin** is a cryIA(b) C-terminalprotoxin portion having a cryIA(b) sequence.

7. A recombinant host transformed to express a **chimeric** Bacillus **thuringiensis** **toxin** comprising a cryIC core N-terminal toxin portion and a cryIA(b) C-terminal protoxin portion.

US PAT NO: 5,583,036 [IMAGE AVAILABLE] L6: 3 of 21
DETDESC:DETD(97)
The . . . vector pCIB10 [Rothstein et al., Gene 53 153-161 (1987) incorporated herein by reference] into which had been inserted the following **chimeric** Bacillus **thuringiensis** **endotoxin** genes ("BT Genes"):

US PAT NO: 5,545,565 [IMAGE AVAILABLE] L6: 4 of 21
SUMMARY:BSUM(2)
This . . . the use of genetic engineering techniques in the modification of plants. More particularly, it concerns introduction and integration of a **chimeric** gene coding for a polypeptide **toxin** produced by Bacillus **thuringiensis** or having substantial sequence homology to a toxin gene described below in plant cells and obtaining an insect controlling level. . .

BSUM(12)
It is one object of this invention to provide novel **chimeric** genes coding for the polypeptide **toxin** produced by Bacillus **thuringiensis**, or coding for a polypeptide toxin having substantial sequence homology to a **toxin** gene described herein. The **chimeric** genes' plant regulatory sequences direct expression in transformed plant cells.

US PAT NO: 5,527,883 [IMAGE AVAILABLE] L6: 5 of 21
ABSTRACT:
Bacillus **thuringiensis** endotoxin expression in Pseudomonads can be improved by modifying the gene encoding the Bacillus **thuringiensis** **endotoxin**. **Chimeric** genes are created by replacing the segment of the Bacillus **thuringiensis** gene encoding a native protoxin with a segment encoding a different protoxin. Exemplified herein is the **cryIF**/**cryI**(b) **chimera** wherein the native **cryIF** protoxin segment has been substituted by the cryIA(b) protoxin segment, to yield improved expression of the cryIF toxin in Pseudomonads.. . .

SUMMARY:BSUM(6)
The . . . Natl. Acad. Sci. U.S.A. 78:2893-2897). U.S. Pat. No. 4,448,885 and U.S. Pat. No. 4,467,036 both disclose the expression of B.t. **crystal** **protein** in E. coli. **Hybrid** B.t. crystal proteins have been constructed that exhibit increased toxicity and display an expanded host range to a target pest. See U.S. Pat. Nos. 5,128,130 and 5,055,294. U.S. Pat. Nos. 4,797,276 and 4,853,331 disclose B. **thuringiensis** strain tenebrionis (a.k.a. M-7, a.k.a. B.t. san diego) which can be used to control coleopteran pests in various environments. U.S.. . . Pat. No. 4,849,217 discloses B.t. isolates which have activity against the alfalfa weevil. U.S. Pat. No. 5,208,077 discloses coleopteran-active Bacillus **thuringiensis** isolates. U.S. Pat. No. 5,151,363 and U.S. Pat. No. 4,948,734 disclose certain isolates of B.t. which have activity against nematodes.. . .

We claim:
1. An isolated polynucleotide molecule comprising a nucleotide sequence encoding a Bacillus **thuringiensis** toxin wherein said Bacillus **thuringiensis** **toxin** is a **chimeric** **toxin** comprising a **cryIF** core N-terminal toxin portion and a heterologous protoxin portion from a cryIA(b) or a **cryIA**(c)/**cryIA**(b) **chimeric** **toxin**.

2. The isolated polynucleotide molecule, according to claim 1, comprising a nucleotide sequence encoding a **chimeric** Bacillus **thuringiensis** **toxin** of approximately 1150 to 1200 amino acids, wherein said toxin comprises a cryIF core N-terminal sequence of at least about. . .

15. A substantially pure **chimeric** Bacillus **thuringiensis** **toxin** comprising a **cryIF** core N-terminal toxin portion and a heterologous C-terminal protoxin portion from a cryIA(b) **toxin** or **cryIA**(b)/**cryIA**(c) **chimeric** **toxin**.

16. The **chimeric** Bacillus **thuringiensis** **toxin**, according to claim 15, having approximately 1150 to 1200 amino acids, wherein said toxin comprises a cryIF core N-terminal sequence. . .

17. The **chimeric** Bacillus **thuringiensis** **toxin**, according to claim 16, wherein the transition from cryIF core N-terminal toxin portion to heterologous protoxin portion occurs after the. . .

18. The **chimeric** Bacillus **thuringiensis** **toxin**, according to claim 17, wherein said core toxin portion comprises the first about 601 amino acids of a cryIF toxin. . .

21. The **chimeric** Bacillus **thuringiensis** **toxin**, according to claim 15, comprises an amino acid sequence shown in FIG. 9.

US PAT NO: 5,518,897 [IMAGE AVAILABLE] L6: 6 of 21
DETDESC:DETD(5)
The present invention involves direct translational fusion, as opposed to transcriptional fusion, between the cyanobacterial cpcB and B. **thuringiensis** subsp. israelensis **cryIVD** genes. Such **fusion** may be explained as follows: the DNA sequence of any gene can be divided into two portions. First, the protein. . .

DETD(8)
In . . . a restriction site at the exact location required to produce an in-frame translational fusion between the cyanobacterial cpcB and B. **thuringiensis** subsp. israelensis cryIVD gene. A translational cpcB-**cryI** gene **fusion** sequence is shown in FIG. 4.

DETD(33)
A . . . extracts of PR-6 cells carrying plasmid pAQE19.DELTA.Sal and the finding that this polypeptide retains the antigenic integrity of theB. **thuringiensis** subsp. israelensis cryIVD protein indicate that these cyanobacterial cells are in fact expressing the cpcB-**cryIVD** gene **fusion** provided by the presence of plasmid pAQRM56.

US PAT NO: 5,508,264 [IMAGE AVAILABLE] L6: 7 of 21
ABSTRACT:
Disclosed are compositions and processes for controlling lepidopteran pests. These compositions comprise synergistic combinations of a **CryIF** **chimeric** and **CryIA**(c) **chimeric** Bacillus **thuringiensis** .delta.-**endotoxin**. These compositions have been found to exhibit excellent activity against lepidopteran pests.

SUMMARY:BSUM(6)
The . . . Acad. Sci. USA 78:2893-2897). U.S. Pat. No. 4,448,885 and U.S. Pat. No. 4,467,036 both disclose the expression of a B.t. **crystal** **protein** in E. coli. **Hybrid** B.t. **crystal** **protein** genes have been constructed that exhibit increased toxicity and display an expanded host range to a target pest. See U.S. Pat. Nos. 5,128,130 and 5,055,294. U.S. Pat. Nos. 4,797,276 and 4,853,331 disclose B. **thuringiensis** strain san diego (a.k.a. B.t. tenebrionis, a.k.a. M-7) which can be used to control coleopteran pests in various environments. U.S.. . .

BSUM(14)
The subject invention concerns the discovery of advantageous increased activity against lepidopteran pests achieved by the combination of two Bacillus **thuringiensis** (B.t.) .delta.-**endotoxin** proteins. More specifically, a **CryIF** **chimeric** **toxin** combined with a **CryIA**(c) **chimeric** **toxin** act in synergy to yield unexpected enhanced toxicity to lepidopteran pests.

US PAT NO: 5,495,071 [IMAGE AVAILABLE] L6: 8 of 21
ABSTRACT:

. . . toxicity to Coleopteran insects. In yet another aspect, the present invention embraces bacterial cells and plant transformation vectors comprising a **chimeric** plant gene encoding a Coleopteran **toxin** protein of Bacillus **thuringiensis**.

JS PAT NO: 5,424,409 [IMAGE AVAILABLE] L6: 9 of 21
SUMMARY:BSUM(9)
n a further aspect, our invention comprises recombinant DNA coding for an insecticidally-active Bacillus **thuringiensis** **endotoxin** which is a **chimera** derived from sequences from at least two separate Bacillus **thuringiensis** genes. The molecular weight of the chimera may be of the order of 110,000 Daltons. Preferably the link or links. . . such genes. In a more specific aspect, our invention comprisesrecombinant DNA coding for an insecticidally-active form of the Bacillus **thuringiensis** endotoxin comprising the rst 1692 basepairs (564 amino acid codons) of the amino-terminal coding region from a 5.3-type endotoxin gene. . .

)ETDESC:DETD(22)
. . . --
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80 2 --
--
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--
--
.3-type **endotoxin**
pJH1 90 1 --
--
--
--
--
--
*Chimeric** **endotoxin**
pJH2 90 1 --
--
--
--
--
--

*carries an endotoxin gene from B. **thuringiensis** HD73
E = early
L = late

JS PAT NO: 5,422,120 [IMAGE AVAILABLE] L6: 10 of 21
)ETDESC:DETD(10)
. . . other avermectins
trazine
ndane
lichlorvos
limethoate
varfarin
,p'-DDD
,p'-DDE
ICH
)MDT
ldrin
lieldrin
Vdicarb
EDB
)CP
)BCP
imazine
yanazine
Bacillus **thuringiensis** toxin
Bacillus **thuringiensis** var. kurstaki
bis(tri-n-butyltin)oxide (TBTO)
)ther organochlorine pesticides
Proteins and Glycoproteins
ymphokines
nterleukins - 1, 2, 3, 4, 5, 6, 7,. . . basic protein
collagen
ibronectin
aminin
)ther proteins made by recombinant DNA technology
rythropoietin
L-3/GM-CSF fusion proteins
Monoclonal antibodies
Polyclonal antibodies
ntibody-**toxin** **fusion** proteins
ntibody radionuclide conjugate
nterferons
Fragments and peptide analogs, and analogs of fragment of proteins, peptides and glycoproteins.
Epidermal growth. . .

JS PAT NO: 5,350,689 [IMAGE AVAILABLE] L6: 11 of 21
)ETDESC:DETD(256)
Example 6a: Construction of pTOX, Containing a **Chimeric** Gene Encoding the Insecticidal **Toxin** Gene of Bacillus **thuringiensis** var tenebrionis

JS PAT NO: 5,317,096 [IMAGE AVAILABLE] L6: 12 of 21
SUMMARY:BSUM(2)
This . . . the use of genetic engineering techniques in the modification of plants. More particularly, it concerns introduction and integration of a **chimeric** gene coding for a polypeptide **toxin** produced by Bacillus **thuringiensis** or having substantial sequence homology to a toxin gene described below in plant cells and obtaining an insect controlling level. . .

SUM(12)
t is one object of this invention to provide novel **chimeric** genes coding for the polypeptide **toxin** produced by Bacillus**thuringiensis**, or coding for a polypeptide toxin having substantial sequence homology to a **toxin** gene described herein. The **chimeric** genes' plant regulatory sequences direct expression in transformed plant cells.

JS PAT NO: 5,306,628 [IMAGE AVAILABLE] L6: 13 of 21
)ETDESC:DETD(25)
According to a preferred embodiment of the invention, DNA sequences encoding B. **thuringiensis** .delta.-endotoxins and the gp64 viral membrane glycoprotein of ACNPV are operably linked, and the combined DNA sequence is expressed in host organisms to produce **chimeric** Bt/gp64 **chimeric** **toxin** proteins.

)ETD(61)
One . . . for delta endotoxins from strains which are toxic to lepidopterans and coleopteran beetles. We have chosen Coleopteran BT toxin Bacillus **thuringiensis** tenebrionis, Btt) over Lepidopteran BT toxin for several reasons. One among them is, since the gp64 is from a virus which infects exclusively lepidopteran hosts, when fused with the coleopteran toxin, it will be easier to assay the **chimeric** **toxin** protein for its newly acquired toxicity against lopidopteran larvae (Trichoplusia ni). For obtaining the gone coding for the coleopteran toxin,. . . screen the colonies of Btt-pUC13 recombinant library. Total DNA (both chromosomal and plasmid) was isolated from the bacterial strain Bacillus **thuringiensis** tenebrionis (Btt). (This strain was obtained from Safer Inc. solated bacterial DNA was then digested with the restriction enzyme HindIII. . .

JS PAT NO: 5,290,914 [IMAGE AVAILABLE] L6: 14 of 21
SUMMARY:BSUM(7)
The . . . gut cell recognition ("binding") protein to direct the cytotoxic agent to the host target. Details for the construction of a **hybrid** B.t. **toxin** are disclosed. The cytotoxic agent is an ADP-ribosylating enzyme. For example, the cytotoxic agent can be the A fragment of. . . with a synthetic DNA linker region to which a gene encoding the insect gut epithelial cell recognition portion of Bacillus **thuringiensis** var. kurstaki HD-73 is ligated.

)ETDESC: DETD(89)
Construction of a **Hybrid** **Toxin** Using NPV Fusogenic Protein to Replace Bacillus **thuringiensis** Recognition Protein

)ETD(90)
Construction . . . open reading frame that codes for the protein. The DNA coding for the recognition fusogen can be cloned into the **hybrid** **toxin** construct in place of the B. **thuringiensis** recognition sequence using techniques described frequently.

JS PAT NO: 5,254,799 [IMAGE AVAILABLE] L6: 15 of 21
SUMMARY:BSUM(3)
This . . . the use of genetic engineering techniques in the modification of plants. More particularly, it concerns introduction and integration of a **chimeric** gene coding for a polypeptide **toxin** produced by Bacillus **thuringiensis** or having substantial equence homology to a toxin gene described below in plant cells and obtaining aninsect controlling level. . .

SUM(13)
. is one object of this invention to provide novel **chimeric** genes coding for the polypeptide **toxin** produced by Bacillus **thuringiensis**, or coding for a polypeptide toxin having substantial sequence homology to a **toxin** gene described herein. The *chimeric** genes' plant regulatory sequences direct expression in transformed plant cells.

JS PAT NO: 5,244,802 [IMAGE AVAILABLE] L6: 16 of 21
)ETDESC:DETD(88)
The . . . T-DNA vector pCIB10 (Rothstein et al, Gene 53:153-161 (198) incorporated herein by reference into which had been inserted the following **chimeric** Bacillus **thuringiensis** **endotoxin** genes ("BT Genes").

JS PAT NO: 5,177,308 [IMAGE AVAILABLE] L6: 17 of 21
ABSTRACT:
Transgenic plants have been created which express an insect-specific **toxin** from a scorpion. The **chimeric** inheritable trait produced conditions of toxicity in the plant cells of toxicity to certain insects upon ingestion of plant tissues. The inheritable trait has also been cross-bred to plants transgenic to the Bacillus **thuringiensis** delta-endotoxin to produce plants having two independent insect-specific toxin traits. Insect feeding trails revealed additive toxic effects. A generalized approach. . .

JS PAT NO: 5,143,905 [IMAGE AVAILABLE] L6: 18 of 21
)ETDESC:DETD(26)
According to a preferred embodiment of the invention, DNA sequences ncoding B. **thuringiensis** .delta.-endotoxins and the gp64 viral membrane glycoprotein of AcNPV are operably linked, and the combined DNA sequence is expressed in host organisms to roduce **chimeric** Bt/gp64 **chimeric** **toxin** proteins.

)ETD(62)
One . . . for delta endotoxins from strains which are toxic to lepidopterans and coleopteran beetles. We have chosen Coleopteran BT toxin Bacillus **thuringiensis** tenebrionis, Btt) over Lepidopteran BT toxin for several reasons. One among them is, since the gp64 is from a virus which infects exclusively lepidopteran hosts, when fused with the coleopteran toxin, it will be easier to assay the **chimeric** **toxin** protein for its newly acquired toxicity against lepidopteran larvae(Trichoplusia ni). For obtaining the gene oding for the coleopteran toxin, Bacillus **thuringiensis** tenebrionis (Btt) was obtained from Safer Inc., Newton, Mass. utilizing the published sequence of Btt protein (Hofte, H. et al.,. . . for PCR were used as probes to screen the colonies chromosomal and lasmid) was isolated from the bacterial strain Bacillus **thuringiensis** tenebrionis (Btt). (This strain was obtained from Safer Inc. Isolated bacterial DNA was then digested with the restriction enzyme HindIII. . .

20. The method of claim 19 wherein said **chimeric** protein comprises a **crystal** **protein** of Bacillus hurinoiensis (B. **thuringiensis**) or a fragment thereof having insecticidal activity and a surface glycoprotein of the extracellular form of a nuclear olyhedrosis. . .

JS PAT NO: 5,128,130 [IMAGE AVAILABLE] L6: 19 of 21
SUMMARY:BSUM(5)
Specifically, the invention comprises a novel **hybrid** delta **endotoxin** gene comprising part of the B. **thuringiensis** var. kurstaki HD-73 toxin gene and part of the toxin gene from B. **thuringiensis** var. kurstaki strain HD-1. This hybrid gene was inserted nto a suitable transfer vector which was then used to transform. . .

)ETDESC:DETD(2)
The novel **hybrid** **toxin** gene of the subject invention comprises part of the B. **thuringiensis** var. kurstaki HD-73 toxin gene and part of a B. **thuringiensis** var. kurstaki strain HD-1 toxin gene. In general, the B.t.k. HD-73 gene portion was initially ombined with DNA segments derived. . .

JS PAT NO: 5,071,654 [IMAGE AVAILABLE] L6: 20 of 21
SUMMARY:BSUM(21)
n a preferred embodiment of this invention, the relative toxicities of Bacillus **thuringiensis**-type protein endotoxins in target insects may be evaluated by the in vitro method of (i) combining insect midgut brush order. . . introducing a Bt-type protein endotoxin, in activated form, into contact with the hybrid phospholipid bilayer, so as to bind the **endotoxin** into the **hybrid** phospholipid bilayer; (iii) ontacting one side of the **endotoxin**-treated **hybrid** phospholipid bilayer with an aqueous solution containing a monovalent cation to create an ion concentration gradient across the bilayer, at a temperature from about 15.degree. C. to 35.degree. C.; (iv) measuring the monovalent cation flow across the **endotoxin**-treated **hybrid** phospholipid bilayer; and (v) comparing he cation flow for the **endotoxin**-treated **hybrid** phospholipid bilayer with that of a control, selected from an **endotoxin**-free **hybrid** phospholipid bilayer or an otherwise identical hybrid phospholipid bilayer treated with a second Bt-type protein endotoxin n lieu of the. . .

2. An in vitro method for evaluating the relative toxicities of Bacillus **thuringiensis**-type protein endotoxins in target insects, which comprises (i) combining insect midgut brush border from a specific target insect and a. . . introducing a Bt-type protein ndotoxin, in activated form, into contact with the hybrid phospholipid bilayer, so as to bind the **endotoxin** into the **hybrid** phospholipid bilayer; (iii) contacting one side of the **endotoxin**-treated **hybrid** phospholipid bilayer with an aqueous solution ontaining a monovalent cation to create an ion concentration gradient across the bilayer, at a temperature from about 15.degree. C. to 35 degree. C.; (iv) measuring the monovalent cation flow across the **endotoxin**-treated **hybrid** phospholipid bilayer; and v) comparing the cation flow for the **endotoxin**-treated **hybrid** phospholipid bilayer with that of a control, selected from an **endotoxin**-free **hybrid** phospholipid bilayer or an otherwise identical hybrid phospholipid bilayer treated with a second Bt-type rotein endotoxin in lieu of the. . .

JS PAT NO: 5,055,294 [IMAGE AVAILABLE] L6: 21 of 21
TITLE: **Chimeric** Bacillus **thuringiensis** **crystal** **protein** gene comprising HD-73 and Berliner 1715 toxin genes, transformed and expressed in Pseudomonas fluorescens

SUMMARY:BSUM(5)
Specifically, the invention comprises a novel **hybrid** delta **endotoxin** gene comprising part of the B. **thuringiensis** var. kurstaki strain HD-73 toxin gene and part of the toxin gene from B. **thuringiensis** var. **thuringiensis** strain Berliner 1715 (DNA :305-314, 1986). This hybrid gene was inserted into a suitable transfer vector which was then used. . .

)ETDESC:DETD(2)
The novel **hybrid** **toxin** gene of the subject invention comprises part of the B. **thuringiensis** var. kurstaki strain HD-73 toxin gene and part of a B. **thuringiensis** var. **thuringiensis** strain Berliner 1715 toxin gene. In general, the B.t.k. HD-73 gene portion was initially combined with DNA segments derived from. . .

-7
1. 5,625,136, Apr. 29, 1997, Synthetic DNA sequence having enhanced insecticidal activity in maize; Michael G. Koziel, et al., 800/205; 435/69.1, 172.3; 536/23.1, 23.71; 800/250, DIG.50 [IMAGE AVAILABLE]

2. 5,608,142, Mar. 4, 1997, Insecticidal cotton plants; Kenneth A. Barton, et al., 800/205; 435/320.1; 800/255, DIG.27 [IMAGE AVAILABLE]

3. 5,567,862, Oct. 22, 1996, Synthetic insecticidal crystal protein gene; Michael J. Adang, et al., 800/205; 435/69.1, 418; 800/250 [IMAGE AVAILABLE]

4. 5,567,600, Oct. 22, 1996, Synthetic insecticidal crystal protein gene; Michael J. Adang, et al., 536/23.71; 435/69.1, 172.3 [IMAGE AVAILABLE]

5. 5,530,195, Jun. 25, 1996, Bacillus **thuringiensis** gene encoding a toxin active against insects; Vance C. Kramer, et al., 800/205; 424/93.2; 435/69.1, 235.1, 252.3, 252.31, 252.34, 320.1; 514/12; 530/350; 536/23.71; 800/DIG.56 [IMAGE AVAILABLE]

6. 5,516,693, May 14, 1996, Hybrid gene incorporating a DNA fragment containing a gene coding for an insecticidal protein, plasmids, transformed cyanobacteria expressing such protein and nethod for use as a biocontrol agent; Mark A. Vaeck, et al., 435/320.1, 69.7, 172.3, 252.33; 536/23.4, 23.71 [IMAGE AVAILABLE]

7. 5,461,032, Oct. 24, 1995, Insecticidally effective peptides; Karen J. Krapcho, et al., 514/12; 435/69.1 [IMAGE AVAILABLE]

8. 5,460,963, Oct. 24, 1995, Plants transformed with a DNA sequence from Bacillus **thuringiensis** lethal to Lepidoptera; Johan Botterman, et al., 435/172.3, 71.3, 320.1, 411, 414, 418; 530/350; 536/23.71; 800/205, DIG.43, DIG.44 [IMAGE AVAILABLE]

9. 5,457,178, Oct. 10, 1995, Insecticidally effective spider toxin; John R. H. Jackson, et al., 530/350 [IMAGE AVAILABLE]

10. 5,441,934, Aug. 15, 1995, Insecticidally effective peptides; Karen J. Krapcho, et al., 514/12; 424/405, 538; 435/69.1, 172.3; 530/300, 324, 345 [IMAGE AVAILABLE]

11. 5,441,884, Aug. 15, 1995, Bacillus **thuringiensis** transposon TN5401; James A. Baum, 435/252.31; 424/93.2; 435/252.3, 252.33, 320.1; 536/23.1, 23.2, 23.7, 24.1 [IMAGE AVAILABLE]

12. 5,382,429, Jan. 17, 1995, Bacillus **thuringiensis** protein toxic to coleopteran insects; William P. Donovan, et al., 424/93.461, 195.1; 435/71.3, 172.3, 252.1, 252.31; 514/12; 530/350, 820 IMAGE AVAILABLE]

13. 5,380,831, Jan. 10, 1995, Synthetic insecticidal crystal protein gene; Michael J. Adang, et al., 536/23.71; 435/69.1, 172.3; 800/205 [IMAGE AVAILABLE]

14. 5,378,625, Jan. 3, 1995, Bacillus **thuringiensis** cryIIIC, (b) protein toxic to coleopteran insects; William P. Donovan, et al., 435/252.5; 424/93.2, 93.461; 435/69.1, 252.3, 320.1; 514/2, 12; 530/350; 536/22.1, 23.1, 23.7, 23.71 [IMAGE AVAILABLE]

15. 5,372,943, Dec. 13, 1994, Lipid microemulsions for culture media; Duane Inlow, et al., 435/404; 252/302; 428/402.2 [IMAGE AVAILABLE]

16. 5,349,124, Sep. 20, 1994, Insect-resistant lettuce plants; David A. Fischhoff, et al., 800/205; 424/93.21; 435/418; 800/DIG.13 [IMAGE AVAILABLE]

17. 5,338,544, Aug. 16, 1994, CryIIB protein, insecticidal compositions nd methods of use thereof; William P. Donovan, 424/93.2, 93.461; 435/69.1, 252.31; 514/2; 530/350 [IMAGE AVAILABLE]

18. 5,264,364, Nov. 23, 1993, Bacillus **thuringiensis** cryIIIc(B) toxin gene and protein toxic to coleopteran insects; Willam P. Donovan, et al., 435/252.5, 6, 69.1, 252.3, 320.1; 536/22.1, 23.1, 23.2, 23.7, 23.71 [IMAGE AVAILABLE]

19. 5,250,515, Oct. 5, 1993, Method for improving the efficacy of insect toxins; Roy L. Fuchs, et al., 514/12; 424/93.461, 195.1; 530/370, 379 [IMAGE AVAILABLE]

20. 5,187,091, Feb. 16, 1993, Bacillus **thuringiensis** cryIIIC gene encoding toxic to coleopteran insects; William P. Donovan, et al., 435/418; 424/93.461; 435/172.3, 252.3, 252.31, 320.1; 536/23.71, 24.32; 935/98 [IMAGE AVAILABLE]

21. 5,110,905, May 5, 1992, Activated Bacillus thuringienses delta-**endotoxin** produced by an engineered **hybrid** gene; Daniel P. Witt, et al., 530/350; 435/69.1, 71.1 [IMAGE AVAILABLE]

22. 5,104,974, Apr. 14, 1992, Bacillus **thuringiensis** coleopteran-active toxin; August J. Sick, et al., 530/350; 435/69.1, 71.1, 172.1, 172.3, 252.3, 254.2, 254.21, 320.1, 822, 911, 946; 530/825; 536/23.71; 935/6, 9, 22, 59, 60, 64, 66, 68, 72, 73, 74, 75 [IMAGE AVAILABLE]

23. 5,073,632, Dec. 17, 1991, CryIIB crystal protein gene from Bacillus **thuringiensis**; William P. Donovan, 536/23.71; 435/172.3; 536/24.1 [IMAGE AVAILABLE]

24. 5,024,947, Jun. 18, 1991, Serum free media for the growth on insect cells and expression of products thereby; Duane Inlow, et al., 435/404, 70.1 [IMAGE AVAILABLE]

25. 4,996,155, Feb. 26, 1991, Bacillus **thuringiensis** gene encoding a coleopteran-active toxin; August J. Sick, et al., 424/93.2, 93.21; 435/69.1, 71.1, 172.1, 172.3, 252.3, 252.5, 254.11, 254.2, 254.21, 320.1, 822, 911, 946; 536/23.71, 24.2; 935/6, 9, 22, 59, 60, 64, 66, 68, 72, 73, 74, 75 [IMAGE AVAILABLE]

26. H 875, Jan. 1, 1991, Toxin-encoding nucleic acid fragments derived from a Bacillus **thuringiensis** subsp. israelensis gene; David J. Ellar, et al., 435/252.31, 69.1, 172.3, 252.5, 832; 530/350, 358; 536/23.7, 23.71; 935/27, 60 [IMAGE AVAILABLE]

27. 4,933,288, Jun. 12, 1990, Use of a modified soluble Pseudomonas exotoxin A in immunoconjugates; I. Lawrence Greenfield, 435/252.3, 69.1, 69.5, 172.3, 252.8, 320.1; 536/23.2, 23.7, 24.1; 935/23, 38, 48 [IMAGE AVAILABLE]

US PAT NO: 5,567,862 [IMAGE AVAILABLE] L7: 3 of 27

ABSTRACT:

Synthetic Bacillus **thuringiensis** toxin genes designed to be expressed in plants at a level higher than naturally-occurring Bt genes are provided. These genes utilize codons preferred in highly expressed monocot or dicot proteins.

SUMMARY:BSUM(2)

This invention relates to the field of bacterial molecular biology and, in particular, to genetic engineering by recombinant technology for the purpose of protecting plants from insect pests. Disclosed herein are the chemical synthesis of a modified crystal protein gene from Bacillus **thuringiensis** var. tenebrionis (Btt), and the selective expression of this synthetic insecticidal gene. Also disclosed is the transfer of the cloned synthetic gene into a host microorganism, rendering the organism capable of producing, at improved levels of expression, a protein having toxicity to insects. This invention facilitates the genetic engineering of bacteria and plants to attain desired expression levels of novel toxins having agronomic value.

BSUM(4)

thuringiensis (Bt) is unique in its ability to produce, during the process of sporulation, proteinaceous, crystalline inclusions which are found to be highly toxic to several insect pests of agricultural importance. The crystal proteins of different Bt strains have a rather narrow host range and hence are used commercially as very selective biological insecticides. Numerous strains of Bt are toxic to lepidopteran and dipteran insects. Recently two subspecies (or varieties) of Bt have been reported to be pathogenic to coleopteran insects: var. tenebrionis (Krieg et al. (1983) Z. Angew. Entomol. 96:500-508) and var. san diego (Herrnstadt et al. (1986) Biotechnol. 4:305-308). Both strains produce flat, rectangular crystal inclusions and have a major crystal component of 64-68 kDa (Herrnstadt et al. Supra; Bernhard (1986) FEMS Microbiol. Lett. 33:261-265).

BSUM(7)

Chimeric **toxin** genes from several strains of Bt have been expressed in plants. Four modified Bt2 genes from var. berliner 1715, under the control of the 2' promoter of the Agrobacterium TR-DNA, were transferred into tobacco plants (Vaeck et al. (1987) Nature 328:33-37). Insecticidal levels of toxin were produced when truncated genes were expressed in transgenic plants. However, the steady state mRNA levels in the transgenic plants were so low that they could not be reliably detected in Northern blot analysis and hence were quantified using ribonuclease protection experiments. Bt mRNA levels in plants producing the highest level of protein corresponded to :apprxeq.0.0001% of the poly(A).sup.+ mRNA.

We claim:

1. A plant cell comprising a heterologous modified structural gene derived from a Bacillus **thuringiensis** gene encoding a pesticidal protein toxin, said plant cell produced by the steps of (a) analyzing the coding sequence of a gene derived from a Bacillus **thuringiensis** which encodes a pesticidal protein toxin; (b) a portion of said coding sequence to yield a modified structural gene which contains a greater number of codons preferred by said plant cell than did said coding sequence prior to modification, said modification comprising reducing the number of codons having CG in codon positions II and III in a region between plant polyadenylation signals in said coding sequence; (c) inserting said modified structural gene into the genome of a plant cell; and (d) maintaining said plant cell under conditions suitable to allow replication of said plant cell to produce additional plant cells having said modified structural gene the genome of said additional plant cells, wherein said modified structural gene is expressed to produce a pesticidal protein toxin.

5. A method of producing a pesticidal protein comprising the steps of (a) introducing into a host plant cell a heterologous modified structural gene derived from a Bacillus **thuringiensis** gene wherein the DNA coding sequence of the Bacillus **thuringiensis** gene has been modified to contain a greater number of codons preferred by said plant cell than did said coding sequence prior to modification, said modification comprising reducing the number of codons having CG in codon positions II and III in a region between plant polyadenylation signals in said coding sequence; and (b) maintaining said plant cell under conditions suitable to allow replication of said plant cell to produce additional plant cells having said modified structural gene in the genome of said additional plant cells, wherein said modified structural gene is expressed to produce a pesticidal protein toxin.

7. A plant cell comprising a heterologous modified structural gene derived from a Bacillus **thuringiensis** gene encoding a pesticidal protein toxin, said plant cell produced by the steps of (a) analyzing the coding sequence of a gene derived from a Bacillus **thuringiensis** which encodes a pesticidal protein toxin; (b) modifying a portion of said coding sequence to yield a modified structural gene which has a frequency of codon usage which more closely resembles the frequency of codon usage of genes native to said plant cell than did said coding sequence prior to modification, said modification comprising reducing the number of codons having CG in codon positions II and III in a region between plant polyadenylation signals in said coding sequence;(c) inserting said modified structural gene into the genome of a plant cell; and(d) maintaining said plant cell under conditions suitable to allow replication of said plant cell to produce additional plant cells having said modified structural gene in the genome of said additional plant cells, wherein said modified structural gene is expressed to produce a pesticidal protein toxin.

12. A method of producing a pesticidal protein comprising the steps of (a) introducing into a host plant cell a heterologous modified structural gene derived from a Bacillus **thuringiensis** gene wherein the DNA coding sequence of the Bacillus **thuringiensis** gene has been modified to contain a frequency of codon usage that more closely resembles the frequency of codon usage of genes native to said plant cell than did said coding sequence prior to modification, said modification comprising reducing the number of codons having CG in codon positions II and III in a region between plant polyadenylation signals in said coding sequence; and (b) maintaining said plant cell under conditions suitable to allow replication of said plant cell to produce additional plant cells having said modified structural gene in the genome of said additional plant cells, wherein said modified structural gene is expressed to produce a pesticidal protein toxin.

13. A plant cell comprising a heterologous modified structural gene derived from a Bacillus **thuringiensis** gene encoding a pesticidal protein toxin, said plant cell produced by the steps of (a) analyzing the coding sequence of a gene derived from a Bacillus **thuringiensis** which encodes a pesticidal protein toxin; (b) modifying a portion of said codon sequence to yield a modified structural gene which contains a greater number of codons preferred by said plant cell than did said coding sequence prior to modification,

and wherein said modification results in fewer occurrences of the sequence AATGAA in said modified structural gene than in said coding sequence; (c) inserting said modified structural gene into the genome of a plant cell; and (d) maintaining said plant cell under conditions suitable to allow replication of said plant cell to produce additional plant cells having said modified structural gene in the genome of said additional plant cells, wherein said modified structural gene is expressed to produce a pesticidal protein toxin.

18. A method of producing a pesticidal protein comprising the steps of (a) introducing into a host plant cell a heterologous modified structural gene derived from a Bacillus **thuringiensis** gene wherein the DNA coding sequence of the Bacillus **thuringiensis** gene has been modified to contain a greater number of codons preferred by said plant cell than did said coding sequence prior to modification, and wherein the modification results in fewer occurrences of the sequence AATGAA in said modified structural gene than in said coding sequence; and (b) maintaining said plant cell under conditions suitable to allow replication of said plant cell to produce additional plant cells having said modified structural gene in the genome of said additional plant cells, wherein said modified structural gene is expressed to produce a pesticidal protein toxin.

19. A plant cell comprising a heterologous modified structural gene derived from a Bacillus **thuringiensis** gene encoding a pesticidal protein toxin, said plant cell produced by the steps of (a) analyzing the coding sequence of a gene derived from a Bacillus **thuringiensis** which encodes a pesticidal protein toxin; (b) modifying a portion of said coding sequence to yield a modified structural gene which has a frequency of codon usage which more closely resembles the frequency of codon usage of genes native to said plant cell than did said coding sequence prior to modification, and wherein said modification results in fewer occurrences of the sequence AATGAA in said modified structural gene than in said coding sequence; (c) inserting said modified structural gene into the genome of a plant cell; and (d) maintaining said plant cell under conditions suitable to allow replication of said plant cell to produce additional plant cells having said modified structural gene in the genome of said additional plant cells, wherein said modified structural gene is expressed to produce a pesticidal protein toxin.

24. A method of producing a pesticidal protein comprising the steps of (a) introducing into a host plant cell a heterologous modified structural gene derived from a Bacillus **thuringiensis** gene wherein the DNA coding sequence of the Bacillus **thuringiensis** gene has been modified to contain a frequency of codon usage that more closely resembles the frequency of codon usage of genes native to said plant cell than did said coding sequence prior to modification, and wherein the modification results in fewer occurrences of the sequence AATGAA in said modified structural gene than in said coding sequence; and (b) maintaining said plant cell under conditions suitable to allow replication of said plant cell to produce additional plant cells having said modified structural gene in the genome of said additional plant cells, wherein said modified structural gene is expressed to produce a pesticidal protein toxin.

US PAT NO: 5,567,600 [IMAGE AVAILABLE] L7: 4 of 27

ABSTRACT:

Synthetic Bacillus **thuringiensis** toxin genes designed to be expressed in plants at a level higher than naturally-occurring Bt genes are provided. These genes utilize codons preferred in highly expressed monocot or dicot proteins.

SUMMARY:BSUM(2)

This invention relates to the field of bacterial molecular biology and, in particular, to genetic engineering by recombinant technology for the purpose of protecting plants from insect pests. Disclosed herein are the chemical synthesis of a modified crystal protein gene from Bacillus **thuringiensis** var. tenebrionis (Btt), and the selective expression of this synthetic insecticidal gene. Also disclosed is the transfer of the cloned synthetic gene into a host microoorganism, rendering the organism capable of producing, at improved levels of expression, a protein having toxicity to insects. This invention facilitates the genetic engineering of bacteria and plants to attain desired expression levels of novel toxins having agronomic value.

BSUM(4)

B. **thuringiensis** (Bt) is unique in its ability to produce, during the process of sporulation, proteinaceous, crystalline inclusions which are found to be highly toxic to several insect pests of agricultural importance. The crystal proteins of different Bt strains have a rather narrow host range and hence are used commercially as very selective biological insecticides. Numerous strains of Bt are toxic to lepidopteran and dipteran insects. Recently two subspecies (or varieties) of Bt have been reported to be pathogenic to coleopteran insects: var. tenebrionis (Krieg et al. (1983) Z. Angew. Entomol. 96:500-508) and var. San Diego (Herrnstadt et al. (1986) Biotechnol. 4:305-308). Both strains produce flat, rectangular crystal inclusions and have a major crystal component of 64-68 kDa (Herrnstadt et al. supra; Bernhard (1986) FEMS Microbiol. Lett. 33:261-265).

Chimeric **toxin** genes from several strains of Bt have been expressed in plants. Four modified Bt2 genes from var. berliner 1715,under the control of the 2' promoter of the Agrobacterium TR-DNA, were transferred into tobacco plants (Vaeck et al. (1987) Nature 328:33-37). Insecticidal levels of toxin were produced when truncated genes were expressed in transgenic plants. However, the steady state mRNA levels in the transgenic plants were so low that they could not be reliably detected in Northern blot analysis and hence were quantified using ribonuclease protection experiments. Bt mRNA levels in plants producing the highest level of protein corresponded to .apprxeq.0.0001% of the poly(A).sup.+ mRNA.

We claim:

1. A method of designing a synthetic Bacillus **thuringiensis** gene to be more highly expressed in plants, comprising the steps of: (a) analyzing the coding sequence of a gene derived from a Bacillus **thuringiensis** which encodes a pesticidal protein toxin; (b) modifying a portion of said coding sequence to yield a modified sequence which contains a greater number of codons preferred by the intended plant host than did said coding sequence prior to modification, said modification comprising reducing the number of codons having CG in codon positions II and III in a region between plant polyadenylation signals in said coding sequence;(c) inserting said modified sequence into the genome of a plant cell; and (d) maintaining said plant cell under conditions suitable to allow replication of said plant cell to produce additional plant cells having said modified sequence in the genome of said additional plant cells, wherein said synthetic Bacillus **thuringiensis** gene is expressed to produce a pesticidal protein toxin.

2. A DNA coding sequence produced by (a) analyzing the coding sequence of a gone derived from a Bacillus **thuringiensis** which encodes a pesticidal protein toxin; and (b) modifying a portion of said coding sequence to yield a modified sequence which contains a greater number of codons preferred by the intended plant host than did said coding sequence, said modification comprising reducing the number of codons having CG in codon positions II and III in a region between plant polyadenylation signals in said coding sequence.

3. The method of claim 1, wherein at least about 32% of the codons in the coding sequence of the native Bacillus **thuringiensis** gene have been modified to yield said synthetic gene.

4. The method of claim 1, wherein at least about 11% of the nucleotides in the coding sequence of the native Bacillus **thuringiensis** gene have been changed to yield said synthetic gene.

5. The DNA coding sequence of claim 2, wherein at least about 32% of the codons in the coding sequence of the native Bacillus **thuringiensis**gene have been modified to yield said modified sequence.

6. The coding sequence of claim 2, wherein at least about 11% of the nucleotides in the coding sequence of the native Bacillus **thuringiensis** gene have been changed to yield said modified sequence.

7. A method of designing a synthesis Bacillus **thuringiensis** gene to be more highly expressed in plants, comprising the steps of: (a) analyzing the coding sequence of a gene derived from a Bacillus **thuringiensis** which encodes a pesticidal protein toxin; (b) modifying a portion of said coding sequence to yield a modified sequence which has a frequency of codon usage of the plant in which it is to be expressed than did said coding sequence prior to modification, said modification comprising reducing the number of codons having CG in codon positions II and III in a region between plant polyadenylation signals in said coding sequence; (c) inserting said modified sequence into the genome of a plant cell; and (d) maintaining said plant cell under conditions suitable to allow replication of said plant cell to produce additional plant cells having said modified sequence in the genome of said additional plant cells, wherein said synthetic Bacillus **thuringiensis** gene is expressed to produce a pesticidal protein toxin.

8. A DNA coding sequence produced by (a) analyzing the coding sequence of a gene derived from a Bacillus **thuringiensis** which encodes a pesticidal protein toxin; and (b) modifying a portion of said coding sequence to yield a modified sequence which has a frequency of codon usage which more closely resembles the frequency of codon usage of the plant in which it is to be expressed than did said coding sequence, said modification comprising reducing the number of codons having CG in codon positions II and III in a region between plant polyadenylation signals in said coding sequence.

9. The method of claim 7, wherein at least about 32% of the codons in the coding sequence of the native Bacillus **thuringiensis** gene have been modified to yield said synthetic gene.

10. The method of claim 7, wherein at least about 11% of the nucleotides in the coding sequence of the native Bacillus **thuringiensis** gene have been changed to yield said synthetic gene.

11. The DNA coding sequence of claim 8, wherein at least about 32% of the codons in the coding sequence of the native Bacillus **thuringiensis** gene have been modified to yield said modified sequence.

12. The coding sequence of claim 8, wherein at least about 11% of the nucleotides in the coding sequence of the native Bacillus **thuringiensis** gene have been changed to yield said modified sequence.

13. A method of designing a synthesis Bacillus **thuringiensis** gene to be more highly expressed in plants, comprising the steps of: (a) analyzing the coding sequence of a gene derived from a Bacillus **thuringiensis** which encodes a pesticidal protein toxin; (b) modifying a portion of said coding sequence to yield a modified sequence which contains a greater number of codons preferred by the intended plant host than did said coding sequence prior to modification, and wherein said modification results in fewer occurrences of the sequence AATGAA in said modified sequence than in said coding sequence; (c) inserting said modified sequence into the genome of a plant cell; and (d) maintaining said plant cell under conditions suitable to allow replication of said plant cell to produce additional plant cells having said modified sequence in the genome of said additional plant cells, wherein said synthesis Bacillus **thuringiensis** gene is expressed to produce a pesticidal protein toxin.

14. A DNA coding sequence produced by (a) analyzing the coding sequence of a gene derived from a Bacillus **thuringiensis** which encodes a pesticidal protein toxin; (b) modifying a portion of said coding sequence to yield a modified sequence which contain a greater number of codons preferred by the intended plant host than did said coding sequence, and wherein said modification results in fewer occurrences of the sequence AATGAA in said modified sequence than in said coding sequence.

15. The method of claim 13, wherein at least about 32% of the codons in the coding sequence of the native Bacillus **thuringiensis** gene have been modified to yield said synthetic gene.

16. The method of claim 13, wherein at least about 11% of the nucleotides in the coding sequence of the native Bacillus**thuringiensis** gene have been changed to yield said synthetic gene.

17. The DNA coding sequence of claim 14, wherein at least about 32% of the codons in the coding sequence of the native Bacillus **thuringiensis** gene have been modified to yield said modified sequence.

18. The coding sequence of claim 14, wherein at least about 11% of the nucleotides in the coding sequence of the native Bacillus**thuringiensis** gene have been changed to yield said modified sequence.

19. A method of designing a synthetic Bacillus **thuringiensis** gene to be more highly expressed in plants, comprising the steps of: (a) analyzing the coding sequence of a gene derived from a Bacillus **thuringiensis** which encodes a pesticidal protein toxin; b) modifying a portion of said coding sequence to yield a modified sequence which has a frequency of codon usage which more closely resembles the frequency of codon usage of the plant in which it is to be expressed than did said coding sequence prior to modification, and wherein said modification results in fewer occurrences of the sequence AATGAA in said modified sequence than in said coding sequence; (c) inserting said modified sequence into the genome of a plant cell; and (d) maintaining said plant cell under conditions suitable to allow replication of said plant cell to produce additional plant cells having said modified sequence in the genome of said additional plant cells, wherein said synthetic Bacillus **thuringiensis** gene is expressed to produce a pesticidal protein toxin.

20. A DNA coding sequence produced by (a) analog the coding sequence of a gene derived from a Bacillus **thuringiensis** which encodes a pesticidal protein toxin; and (b) modifying a portion of said coding sequence to yield a modified sequence which has a frequency of codon usage which more closely resembles the frequency of codon usage of the plant in which it is to be expressed than did said coding sequence, and wherein said modification results in fewer occurrences of the sequence AATGAA in said modified sequence than in said coding sequence.

21. The method of claim 19, wherein at least about 32% of the codons in the coding sequence of the native Bacillus **thuringiensis** gene have been modified to yield said synthetic gene.

22. The method of claim 19, wherein at least about 11% of the nucleotides codons sequence of the native Bacillus **thuringiensis** gene have been changed to yield said synthetic gene.

23. The DNA coding sequence of claim 20, wherein at least about 32% of the codons in the coding sequence of the native Bacillus **thuringiensis** gene have been modified to yield said modified sequence.

24. The coding sequence of claim 20, wherein at least about 11% of the nucleotides in the coding sequence of the native Bacillus **thuringiensis** gene have been changed to yield said modified sequence.

US PAT NO: 5,461,032 [IMAGE AVAILABLE] L7: 7 of 27
SUMMARY:BSUM(5)
The most widely used microbial pesticides are derived from the bacterium Bacillus **thuringiensis** (hereinafter B.t.). This bacterial agent is used to control a variety of leaf-eating caterpillars, Japanese beetles and mosquitos. U.S. Pat. No. 4,797,279 issued Jan. 10, 1989 to Karamata, et al., discloses hybrid bacterial cells comprising the gene coding for B.t. kurstaki delta-endotoxin and the gene coding for B.t. tenebrionis delta-endotoxin and their preparation. The B.t. hybrids are activeagainst pests susceptible to B.t. kurstaki strains as well as against pests susceptible to B.t. tenebrionis strains. Generally, these hybrids have useful insecticidal properties which are superior to those observed by physical mixtures of the parent strains in terms of level of insecticidal activity, or in terms of spectrum of activity, or both. The insecticidal compositions comprising such microorganisms may be used to combat insects by applying the hybrids in an insecticidally effective amount to the insects or to their environment.

BSUM(6)
Another derivation from the bacterium B.t. was disclosed in European Patent Application, Publication No. 0 325 400 A1, issued to Gilroy and Wilcox. This invention relates to a **hybrid** **toxin** gene which is toxic to lepidopteran insects. Specifically, the invention comprises a **hybrid** delta-**endotoxin** gene comprising part of the B.t. var. kurstaki HD-73 toxin gene and part of the toxin gene from B.t. var. kurstaki strain HD-1. The **hybrid** **toxin** gene (DNA) encoding a protein having activity against lepidopteran insects was disclosed.

BSUM(7)
The bacterium B.t. was also utilized for its insecticidal properties in European Patent Application, Publication No. 0 340 948, issued to Wilcox, et al. This invention concerns hybrid pesticidal toxins which are produced by the fusion of an insect gut epithelial cell recognition region of a B.t. gene to diphtheria **toxin** B chain to prepare a **hybrid** B.t. **toxin** which is active against lepidopteran insects. It was suggested that the hybrid B.t. gene may be inserted into a plantor cloned into a baculovirus to produce a toxin which can be recovered. Alternatively, the host containing the hybrid B.t. gene can be used as an insecticide by direct application to the environment of the targeted insect.

DETDESC:DETD(53)
Furthermore, it is believed the insecticidally effective peptide may be combined with another compound or compounds to produce unexpected insecticidal properties in the transformed plant, containing chimeric genes, expressing the compounds. These other compounds can include protease inhibitors, for example, which have oral toxicity to insects or polypeptides from Bacillus **thuringiensis**. The B. **thuringiensis** protein causes changes in potassium permeability of the insect gut cell membrane and is postulated to generate small pores in the membrane. Other pore-forming proteins could also be used in combination with the insecticidally effective peptides. Examples of such pore-forming proteins are the magainins, the cecropins, the attacins, melittin, gramicidin S, sodium channel proteins and synthetic fragments, the .alpha.-toxin ofStaphylococcus aureus, apolipoproteins and their fragments, alamethicin and a variety of synthetic amphipathic peptides. Lectins which bind to cell membranes and enhance endocytosis are another class of proteins which could be used in combination with the insecticidally effective peptides of this invention to genetically modify plants for insect resistance.

DETD(96)
Various prokaryotic and eukaryotic microbes can be transformed to express a **hybrid** **toxin** gene encoding an insecticidally effective protein by the method taught in European Patent Application 0 325 400.

US PAT NO: 5,457,178 [IMAGE AVAILABLE] L7: 9 of 27
SUMMARY:BSUM(5)
The most widely used microbial pesticides are derived from the bacterium Bacillus **thuringiensis** (hereinafter B.t.). This bacterial agent is used to control a variety of pests, including leaf-eating caterpillars, beetles and mosquitos. U.S. Pat. No. 4,797,279 issued Jan. 10, 1989 to Karamata et al., discloses hybrid bacterial cells comprising the gene coding for B.t. kurstaki delta-endotoxin and the gene coding for B.t. tenebrionis delta-endotoxin and their preparation. The B.t. hybrids are active against pests susceptible to B.t. kurstaki strains as well as against pests susceptible to B.t. tenebrionis strains. Generally, these hybrids have useful insecticidal properties which are superior to those observed by physical mixtures of the parent strains in terms of level of insecticidal activity, or in terms of spectrum of activity, or both. The insecticidal compositions comprising such microorganisms may be used to combat insects by applying the hybrids in an insecticidally effective amount to the insects or to their environment.

BSUM(6)
Another derivation from the bacterium B.t. was disclosed in European Patent Application, Publication No. 0 325 400 A1, issued to Gilroy and Wilcox. This invention relates to a **hybrid** **toxin** gens which is toxic to lepidopteran insects. Specifically, the invention comprises a **hybrid** delta **endotoxin** gens comprising part of the B.t. var. kurstaki HD-73 toxin gens and part of the toxin gens from B.t. var. kurstaki strain HD-1. The **hybrid** **toxin** gens (DNA) encoding a protein having activity against lepidopteran insects was disclosed.

BSUM(7)
The bacterium B.t. was also utilized for its insecticidal properties in European Patent Application, Publication No. 0 340 948, issued to Wilcox, et al. This invention concerns hybrid pesticidal toxins which are produced by the fusion of an insect gut epithelial cell recognition region of a B.t. gene to diphtheria **toxin** B chain to prepare a **hybrid** B.t. **toxin** which is active against lepidopteran insects. It was suggested that the hybrid B.t. gens may be inserted into a plant or cloned into a baculovirus to produce a toxin which can be recovered. Alternatively, the host containing the hybrid B.t. gens can be used as an insecticide by direct application to the environment of the targeted insect.

DETDESC:DETD(52)
Various prokaryotic and eukaryotic microbes can be transformed to express a **hybrid** **toxin** gene encoding an insecticidally effective protein by the method taught in European Patent Application 0 325 400.

US PAT NO: 5,441,934 [IMAGE AVAILABLE] L7: 10 of 27
SUMMARY:BSUM(5)
The most widely used microbial pesticides are derived from the bacterium Bacillus **thuringiensis** (hereinafter B.t.). This bacterial agent is used to control a variety of pests, including leaf-eating caterpillars, beetles and mosquitos. U.S. Pat. No. 4,797,279 issued Jan. 10, 1989 to Karamata et al., discloses hybrid bacterial cells comprising the gene coding for B.t. kurstaki delta-endotoxin and the gene coding for B.t. tenebrionis delta-endotoxin and their preparation. The B.t. hybrids are active against pests susceptible to B.t. kurstaki strains as well as against pests susceptible to B.t. tenebrionis strains. Generally, these hybrids have useful insecticidal properties which are superior to those observed by physical mixtures of the parent strains in terms of level of insecticidal activity, spectrum of activity, or both. The insecticidal compositions comprising such microorganisms may be used to combat insects by applying the hybrids in an insecticidally effective amount to the insects or to their environment.

BSUM(6)
Another derivation from the bacterium B.t. is disclosed in European Patent Application, Publication No. 0 325 400 A1, issued to Gilroy and Wilcox. This invention relates to a **hybrid** **toxin** gene which is toxic to lepidopteran insects. Specifically, the invention comprises a **hybrid** delta **endotoxin** gene comprising part of the B.t. var. kurstaki HD-73 toxin gene and part of the toxin gene from B.t. var. kurstaki strain HD-1. The **hybrid** **toxin** gene (DNA) encoding a protein having activity against lepidopteran insects is disclosed. BSUM(7) The bacterium B.t. has also been utilized for its insecticidal properties as described in European Patent Application, Publication No. 0 340 948, issued to Wilcox, et al. This invention concerns hybrid pesticidal toxins which are produced by the fusion of an insect gut epithelial cell recognition region of a B.t. gene to diphtheria **toxin** B chain to prepare a **hybrid** B.t. **toxin** which is active against lepidopteran insects. It is suggested that the hybrid B.t. gene may beinserted into a plant or cloned into a baculovirus to produce a toxin which can be recovered. Alternatively, the host containing the hybrid B.t. gene can be used as an insecticide by direct application to the environment of the targeted insect.

DETDESC:DETD(89)
Various prokaryotic and eukaryotic microbes can be transformed to express a **hybrid** **toxin** gene encoding an insecticidally effective protein by the method taught in European Patent Application 0 325 400.

US PAT NO: 5,380,831 [IMAGE AVAILABLE] L7: 13 of 27
ABSTRACT:
Synthetic Bacillus **thuringiensis** toxin genes designed to be expressed in plants at a level higher than naturally-occurring Bt genes are provided. These genes utilize codons preferred in highly expressed monocot or dicot proteins.

SUMMARY:BSUM(2)
This invention relates to the field of bacterial molecular biology and, in particular, to genetic engineering by recombinant technology for the purpose of protecting plants from insect pests. Disclosed herein are the chemical synthesis of a modified crystal protein gene from Bacillus **thuringiensis** var. tenebrionis (Btt), and the selective expression of this synthetic insecticidal gene. Also disclosed is the transfer of the cloned synthetic gene into a host microorganism, rendering the organism capable of producing, at improved levels of expression, a protein having toxicity to insects. This invention facilitates the genetic engineering of bacteria and plants to attain desired expression levels of novel toxins having agronomic value.

BSUM(4)
thuringiensis (Bt) is unique in its ability to produce, during the process of sporulation, proteinaceous, crystalline inclusions which are found to be highly toxic to several insect pests of agricultural importance. The crystal proteins of different Bt strains have a rather narrow host range and hence are used commercially as very selective biological insecticides. Numerous strains of Bt are toxic to lepidopteran and dipteran insects. Recently two subspecies (or varieties) of Bt have been reported to be pathogenic to coleopteran insects: var. tenebrionis (Krieg et al. (1983) Z. Angew. Entomol. 99:500-508) and var. san diego (Herrnstadt et al. (1986) Biotechnol. 4:305-308). Both strains produce flat, rectangular crystal inclusions and have a major crystal component of 64-68 kDa (Herrnstadt et al. supra; Bernhard (1986) FEMS Microbiol. Lett. 33:261-265).

SUMMARY:BSUM(7)
"Chimeric" **toxin** genes from several strains of Bt have been expressed in plants. Four modified Bt2 genes from var. berliner 1715, under the control of the 5' promoter of the Agrobacterium TR-DNA, were transferred into tobacco plants (Vaeck et al. (1987) Nature 328:33-37). Insecticidal levels of toxin were produced when truncated genes were expressed in transgenic plants. However, the steady state mRNA levels in the transgenic plants were so low that they could not be reliably detected in Northern blot analysis and hence were quantified using ribonuclease protection experiments. Bt mRNA levels in plants producing the highest level of protein corresponded to .apprxeq.0.0001% of the poly(A).sup.+ mRNA.

We claim:

. A method of designing a synthetic Bacillus **thuringiensis** gene to be more highly expressed in plants, comprising the steps of: analyzing the coding sequence of a gene derived from a Bacillus **thuringiensis** which encodes an insecticidal protein toxin, and modifying a portion of said coding sequence to yield a modified sequence which contains a greater number of codons preferred by the intended plant host than did said coding sequence.

1. A method of designing a synthetic Bacillus **thuringiensis** gene to be more highly expressed in plants, comprising the steps of: analyzing the coding sequence of a gene derived from a Bacillus **thuringiensis** which encodes an insecticidal protein toxin, and modifying a portion of said coding sequence to yield a modified sequence which has a frequency of codon usage which more closely resembles the frequency of codon usage of the plant in which it is to be expressed.

2. The method of claim 11, wherein the modification step comprises the ubstitution of at least one nucleotide in the native Bacillus **thuringiensis** coding sequence.

S PAT NO: 5,250,515 [IMAGE AVAILABLE] L7: 19 of 27
BSTRACT:
method for potentiating the insecticidal activity of a protein toxin of Bacillus **thuringiensis** bacteria is disclosed. A potentiating amount of trypsin inhibitor is co-administered to the insect along with the toxin. Improved insecticidal compositions are also disclosed hich contain an insecticidal amount of a protein toxin of Bacillus **thuringiensis** and a potentiating amount of a trypsin inhibitor.

SUMMARY: BSUM(1)
The present invention relates to insect toxins produced by strains of Bacillus **thuringiensis**. More particularly, the present invention relates to a method for improving the efficacy of such toxins by co-administering an effective amount of a trypsin inhibitor.

BSUM(4)
A totally distinct class of proteins have been isolated from numerous strains of Bacillus **thuringiensis** (B.t.) which also inhibit insect development and have insecticidal activity. The proteincrystalline toxins produced by B.t. represent the major class of proteins used for insect control; Klausner, Bio/Technology 2:408-419. B.t. is a gram-positive, spore forming, soil bacterium which characteristically produces a parasporal crystal protein which accounts for the insecticidal activity. A variety of B.t. strains have been isolated which produce toxins active against a wide range of insects including Lepidopterans, Coleopterans and Dipterans. Numerous Lepidopteran-active strains of B.t. have been isolated and the parasporal crystal proteins analyzed. These proteins are typically encoded as 130 to 140 Kd proteins which are subsequently proteolytically activated in the midgut of the susceptible insect to form the active toxin having a molecular eight of about 65-70 Kd, Aronson, et al., (1968) Microbiol. Rev. 50: 1-24. Crystal/spore preparations of B.t. subspecies kurstaki have been used as commercial insecticides for many years in products such as DIPEL.RTM. (Abbott Laboratories) and THURICIDE.RTM. (Sandoz). These commercial B.t.k. insecticides are effective against more than fifty species of Lepidopteran pests, Wilcox, et al. (1986) Protein Engineering, Inouye and Sarma (Eds.) Academic Press, NY. The toxin produced by B.t. israeliensis, isolated in Israel in1977, has been demonstrated to be toxic to larvae of several Dipteran aquatic insects such as mosquitoes and black flies (EPO Publ. No. 0195285). Recently, B.t. toxins were isolated from B.t. tenebrionis and B.t. san diego which exhibit toxicity against Coleopteran insects; see Herrnstadt et al., 1986, Bio/Technology 4:305-308 and Krieg, et al., 1983, Z. Angew. Entomologie 500-508.

DETDESC:DETD(2)
In its broadest aspect, the present invention provides a method for enhancing the insecticidal activity of the parasporal protein of the soil bacterium Bacillus **thuringiensis**. More particularly, the insecticidal activity of a B.t. toxin is improved by co-administering an effective amount of a trypsin inhibitor. By "insecticidally effective amount" is meant that amount of toxin necessary to cause insect mortality or larval weight reduction and/or delay in development.

DETD(4)
Therefore, in one aspect the present invention provides improved toxin compositions comprising an insecticidally effective amount of a toxinprotein of a Bacillus **thuringiensis** and an effective amount of trypsin inhibitor to enhance the insecticidal activity of the B.t. toxin. The inhibitor is present in a molar ratio versus toxin between 1/10.sup.-2 to 10.sup.6 /1 when the toxin is present at a concentration between and 10.sup.-10 and 10.sup.-7 M. An inhibitor/toxin ratio between about 1/1 and 10.sup.4 /1 is preferred. Those skilled in the art recognize that the potentiating effect due to the presence of inhibitor will vary with the target insect.

DETD(7)
Lepidopteran-type toxins and structural genes encoding such toxins can be obtained from subspecies of Bacillus **thuringiensis** including, but not necessarily limited to, B.t. kurstaki HD-1, B.t. kurstaki HD-73, B.t. sotto, B.t. berliner, B.t. **thuringiensis**, B.t. tolworthi, B.t. dendrolimus, B.t. alesti, B.t. galleriae, B.t. aizawai and B.t. subtoxicus. Dipteran-type toxins and structural genes encoding such toxins can be obtained from subspecies such as B.t. israeliensis. Coleopteran-type toxins and structural genes encoding such toxins can be obtained from subspecies of Bacillus **thuringiensis** including, but not necessarily limited to, B.t. tenebrionis and B.t. san diego. For clarity and brevity of explanation, the present invention will be further described using Lepidoperan-type toxins from B.t. kurstaki HD-1 and HD-73 and a Coleopteran-type toxin from B.t. tenebrionis.

DETD(17)
Numerous methods have been used to purify Lepidopteran-type toxins from B.t. bacteria; Johnson, D. E., (1975) Incidence of Insect Cell Cytolytic Activity Among Bacillus **thuringiensis** serotypes. FEMS Microbiology Lettres 43:121-125; Lecadet, M. M. and Dedonder, R. (1971) Biogenesis of the Crystalline Inclusion of B. **thuringiensis** during sporulation. ur. J. Biochem. 23:282-294; Schesser, J. H., Kramer, K. J. and Bulla, Jr. L. A. (1977) Bioassay for Homogeneous Parasporal Crystal of Bacilus **thuringiensis** using the Tobacco Budworm, Manduca Sexta, Appl. Environ. Microbiol. 33:878-880; Tojo, A. and Aizawa, K. (1983)Dissolution and Degradation of Bacillus **thuringiensis**& Endotoxin by gut juice Protease of the Silkworm Bombyx, Appl. Environ. Microbiol. 45:2:576-580; Nickerson, K. W. and Bulla, Jr. L. A. (1974) Appl. Microbiol. 28:124-128. One method to isolate the toxin from B.t.k. HD-73 bacteria is disclosed by Yamamoto et al., 1983, Arch. of Biochem. & Biophys. 227:1:233-241. The bacteria are grown in a culture medium containing peptonized milk nutrient, glucose, yeast extract, potassium phosphate monobasic and other trace minerals. Fermentation is maintained at 30.degree. C. until almost all cells produce spores and crystals. The cells are lysed and the crystals are harvested by centrifugation at 10,000 g for 2 min. and washed in 1 M NaCl by repeating the centrifugation at least three times to remove bacterial proteases. The mixture of spores and crystals are suspended in water and shaken in a eparatory funnel until foam develops. The crystals in the aqueous layer are separated from the spore-containing foam layer, and this separationby foaming is repeated at least 10 times until almost all spores are removed. The crystals are further purified by isopycnic centrifugation using a sodium bromide (NaBr) density gradient. An aliquot of the crystal suspension is layered on a linear density gradient of NaBr (1.30 to 1.40 g/ml) and centrifuged at 100,000 g for 2 hours. The crystal band is located by examining each band with a phase contrast microscope. The NaBr is removed from the crystals by centrifugation followed by dialysis in water. The purified crystals are lyophilized and stored at -20.degree. C. until used.

DETD(23)
The insecticidal compositions of the present invention comprise a toxin protein(s) from a strain of Bacillus **thuringiensis** and an effective amount of a suitable trypsin inhibitor to enhance the insecticidal activity of the respective toxin protein. In most cases the amount of protease inhibitor will comprise between 0.0000002 and 2.0 wt % of the diet. However, in many cases effective insecticidal enhancement of the toxin can be obtained with inhibitor levels less than 0.02 wt %, levels which are far below the inhibitor levels which exhibit insecticidal activity alone. In many cases it will be possible to use crude preparations of B.t. toxin which comprise sporulated cultures containing the endogenous toxin protein. The inhibitor is present in a molar ratio versus toxin between 1/10.sup.-2 to 10.sup.6 /1 when the toxin is present at a concentration between and 0.sup.-10 and 10.sup.-7 M. An inhibitor/ toxin ratio between about 1/1 and 10.sup.4 /1 is preferred. Those skilled in the art recognize that the potentiating effect due to the presence of inhibitor will vary with the target insect.

DETD(24)
The improved insecticidal compositions may also include a suitable carrier such as vermiculite, silica, etc. The composition may also be dispersed in a polymer to enhance its handling characteristics and enhance its tolerance to degradation due to environment conditions particularly exposure to ultraviolet light. A trypsin inhibitor gene can be engineered for expression in Bacillus **thuringiensis** in order to produce by fermentation a microbial insecticide that contains appropriate levels of both B.t. protein and trypsin inhibitor.

DETD(110)
Using this N-terminal protein sequence information, synthetic DNA probes were designed which were used in the isolation of clones containing the B.t.t. toxin gene. Probes were end-labeled with [.gamma.-.sup.32 P] ATP according to Maniatis (1982), supra. B. **thuringiensis** var. tenebrionis was grown for 6 hours at 37.degree. C. in Spizizen medium (Spizizen, J., 1958, P.N.A.S. USA 44:1072-1078) supplemented with 0.1%yeast extract and 0.1% glucose (SPY) for isolation of total DNA. Total DNA was isolated from B.t.t. by the method of Kronstad (1983), supra. Cells were grown on Luria agar plates for isolation for B.t.t. crystals used in toxicity studies.

DETD(133)
Isolation of DNA sequences encoding the toxin protein of B. **thuringiensis** is well known in the art. The coding sequence from the above-identified subspecies are quite homologous, particularly in the N-terminus region of the coding sequence. This homology is useful in the isolation of other toxin protein coding sequences, since a DNA probe useful in the isolation of B.t. subspecies kurstaki HD-1 as described hereinafter would be useful in the isolation of toxin coding sequences from other subspecies.

DETD(135)
The amino acid sequence of the crystal protein toxin gene isolated from Bacillus **thuringiensis** subspecies kurstaki HD-1 was partially determined according to the method of Hunkapiller et al. (1983) Methods Enzymol. 91:399-413. These sequences were verified using the DNA sequence of the NH.sub.2 -terminal portion of the crystal protein gene disclosed by Wong et al. (1983) J. Biol. Chem. 258:1960-1967. Synthetic oligonucleotide sequences based on an amino acid sequence determined from the crystal protein polypeptide were prepared according to the procedure of Beaucage et al. (1981) Tetrahedron Lett. 22:1859; see also Adams, S. P. et al. (1983) JACS, 105:661-663. The oligonucleotide probes prepared are as shown in Table I below.

DETD(137)
Plasmid DNA from B. **thuringiensis** subspecies kurstaki HD-1 was purified from 1 to 2 liters of culture according to the procedure of Kronstad et al. (1983) J. Bacteriol 154:419-428. All plasmid preparations were banded at least once in CsCl/ethidium bromide gradients. Ptasmids 30 megadaltons and larger in size were preferentially isolated.

DETD(140)
BamHI-restricted pBR328 (100ng), treated with alkaline phosphatase (Boehringer Mannheim) was mixed and ligated with 500 ng of B. **thuringiensis** plasmid DNA restricted with BamHI. CaCl.sub.2 prepared competent E. coli SR200 were transformed and selected by ampicillin resistance and screened for tetracycline sensitivity. Analysis by mini-plasmid prep procedures (Maniatis et al. 1982, Molecular Cloning, A Laboratory Manual, Cold Spring Harbor, N.Y., p. 396) identified two clones which had the correct 16 Kb insert. Southern hybridization analysis with radiolabelled probes from Table I demonstrated that the DNA fragment which contained the sequence hybridizing to the synthetic probe had been sub-cloned. The two plasmids designated pMAP1 and pMAP2, differed only in the orientation of the DNA fragment within the vector.These plasmid constructs produced material cross-reactive to B.t. crystal protein toxin antibody when analyzed according to Western blot procedures (Geshoni et al. 1983, Anal. Biochem. 131:1-15). A restriction map of the inserted B.t. fragment was prepared and four EcoRI (E) sites and three Hind III (H) sites were located between the BamHI (B) sites. This is schematically illustrated as: ##STR12##

DETD(144)
To make a **chimeric** gene encoding the **toxin** protein of B.t. a NcoI site is introduced at the translational initiation codon (ATG) of the DNA encoding the B.t. toxin such that the ATG codon is contained within the NcoI recognition site (CCATGG). DNA sequence analysis of the region of the toxin gene around the initiator codon revealed the sequence: ##STR13## To introduce the desired NcoI site, it was necessary to change the sequence around the ATG from TTATGG to CCATGG. Referring toFIG. 3, a 340 bp DraI-EcoRI fragment which includes the translational initiation region was sub-cloned from pMAP4 between the SmaI and EcoRI sites of the filamentous bacteriophage vector M14mp8. This plasmid was named pMON9732. Single-stranded phage DNA from this construct contains the noncoding strand of the toxin gene sequence.

DETD(146)
An intact toxin gene was constructed which incorporated the NcoI site from the site-specific mutagenesis described above. Referring to FIG. 4, pMAP3 was digested with BamHI and ClaI and a fragment containing the pUC8 vector and the toxin gene from the ClaI site at position 1283 to the PstI site beyond the end of the gene was isolated. A 185 bp fragment extending from the BamHI site was in the mp8 multi-linker to the ClaI site at position 106 was isolated from pMON9733. These two fragments were ligated to create MAP16. pMAP16 contains the NcoI site at the ATG but is missing the segment of the toxin gene between the ClaI sites at 106 and 1283. This ClaI fragment was isolated from pMAP4 and ligated with ClaI digested pMAP16. A plasmid containing this inserted ClaI fragment in the proper orientation to reconstruct a functional toxin gene was identified and designated pMAP17. E. coli containing this plasmid produced a protein of about 134,000 daltons which reacted with antibodies prepared against purified crystal toxin protein from Bacillus **thuringiensis** subspecies kurstaki HD-1 at levels comparable to those produced by E. coli containing pMAP4. coli containing pMAP17 were toxic to the Lepidopteran larvae Manduca sexta.

DETD(147)
To facilitate construction of **chimeric** **toxin** genes in plant transformation vectors, BamHI and BglII sites were introduced justupstream of the NcoI site in the toxin gene. Referring to FIG. 5, plasmid pMON146 was used as a source of a synthetic linker containing restriction sites for BamHI, BglII, XbaI and NcoI as shown: ##STR15## pMON146 was partially digested with PstI and then digested to completion with NcoI, and a 3.5 Kb NcoIPstI fragment was isolated. The 4.5 kb NcoI-PstIfragment containing the entire toxin gene was isolated from pMAP17, and this fragment was ligated with the 3.5 kb pMON146 fragment. A plasmid containing these two fragments was designated MON294. In pMON294 a BamHI and a BglII site are just upstream of the initiation codon for the toxin protein, and a BamHI site is just downstream of the PstI site.

What is claimed is:
. A composition comprising a toxin protein of a Bacillus "thuringiensis" bacteria, which toxin protein exhibits toxicity to Lepidopteran or Coleopteran insects, and a potentiating amount of a trypsin inhibitor which amount of inhibitor is between about 0.000002 and .0 weight percent of the composition and the molar ratio of inhibitor to toxin is in the range of about 1/1 to 104/1.

3. The composition of claim 1 in which the toxin protein is from a source selected from the group consisting of B.t. Kurstaki HD-1, B.t. kurstaki HD-73, B.t. sotto, B.t. berliner, B.t. **thuringiensis**, B.t. tolworthi, B.t. dendrolimus, B.t. alesti, B.t. gallaeriae, B.t. aizawai nd B.t. subtoxicus, B.t. israeliensis, B.t. tenebrionis and B.t. san diego.

IS PAT NO: 5,110,905 [IMAGE AVAILABLE] L7: 21 of 27
ITLE: Activated Bacillus thuringienses delta-**endotoxin** produced by an engineered **hybrid** gene

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USPT	cryig adj cryic or (cryig near3 cryic)	0	L13
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USPT	l7 and l8	49	L9
USPT	l5.ti,ab,clm.	41358	L8
USPT	l6 and l3	153	L7
USPT	l2 same l5	1168	L6
USPT	fus$ or chimer$	182589	L5
USPT	fus$ or chimer$	145777	L4
USPT	l1 same l2	531	L3
USPT	toxin or exotoxin	11583	L2
USPT	thuringiensis	1589	L1

ABSTRACT:
The subject invention is directed to a novel Bacillus **thuringiensis** kurstaki .delta.-endotoxin prepared by use of a novel hybrid gene. This gene is cloned into a novel plasmid which is transformed into a prokaryotic host. The .delta.-endotoxin of the subject nvention is active against Lepidoptera larvae.

SUMMARY:BSUM(2)
Bacillus **thuringiensis**, a spore-forming bacterium of which there are more than 200 naturally occurring variants, produces a rhombic crystal during sporulation. This crystal is toxic upon ingestion to a wide variety of lepidopteran larvae. Many of these susceptible arvae are economically important crop pests. The toxic factor in the crystal is derived from a protein protoxin of molecular weight 130,000 which has been termed the .delta.-endotoxin; the protoxin is not in itself toxic but requires proteolytic processing to yield an active toxin (activated .delta.-endotoxin),and processing normally occurs in the insect gut.

BSUM(3)
Bacillus **thuringiensis** (B.t.) toxin has provided a basis for commercial formulations of insecticide for at least ten years. The active ingredient in these products is dried preparations of sporulated B.t. cells. Included in this dried powder is the rhombic crystal and he viable spore which can regenerate to give rise to vegetative B.t. cells.

BSUM(13)
n 1981, a gene encoding the protoxin from a commercial strain of B.t. was cloned and expressed in E. coli by Schnepf and coworkers (Schnepf, H. E. and Whiteley, H. R. [1981] Proc. Natl. Acad. Sci USA 78: 2893-2897) A U.S. patent was granted on this construction (U.S. Pat. No. 4,448,885). he recombinant plasmid encodes the entire protoxin molecule and the gene is under the control of its natural promoter. Subsequently, a Europeanpatent has been filed by Klier et al on a recombinant protoxin gene from what s presumably a different strain (B. **thuringiensis** 1715) (Klier, A., Rapoport, G., Dedonder, R. [Filing date Apr. 26, 1982] Demande de Brevet Europeen 0 093 062). In neither of these patents is the sequence of the gene or the protein product disclosed It is clear hat the toxin genes in both cases are bounded by undefined sequences of DNA.

BSUM(17)
2. The activated .delta.-**endotoxin** produced directly from the **hybrid** gene is, in essence, a chemical product. The formulation in which it is applied for pest control will contain no viable microorganisms or spores. This constitutes a significant advantage over the commercial preparations presently in use that result in the application of viable spores into the environment.

BSUM(18)
3. The activated .delta.-**endotoxin** produced by the **hybrid** gene is insoluble but is readily extracted into soluble form in aqueous solutions. This can present advantages for application. Insoluble toxin crystals derived from B.t. may present problems with egard to application and coverage. These problems are obviated with a soluble preparation.

BSUM(21)
6. The protein produced by the **hybrid** gene is a preactivated **toxin** and requires no further processing or alteration for full activity. In contrast, the .delta.-endotoxins derived from the natural source as well as those expressed by the recombinant plasmid of others (Schnepf, H. E. and Whiteley, H. R., U.S. Pat. No. 4,448,885);Klier, A., Rapoport, G., Dedonder, R. [Filing date Apr. 26, 1982] Demande de Brevet Europeen 0 093 062) are inactive molecules and require proteolytic processing for activity (Lecadet, M. M. and Dedonder, R. [1967] J. Invert. Pathol. 9:322). Although processing of these protoxins can occur in the insect gut, this preactivation may provide an improvement in speed of kill, an important consideration in the commercial utilization of B.t. toxin.

DETDESC.DETD(2)
A .delta.-endotoxin gene was cloned from a 72 Md plasmid from Bacillus**thuringiensis** var.kurstaki (B.t.k.). Cloning is described in the Examples. The resulting recombinant plasmid, pK15, when transformed into E. coli expressed a protein that reacted with antisera directed toward B.t.k. endotoxin and was toxic to tobacco budworm (TBW) larvae.

DETD(35)
3. **thuringiensis** kurstaki HDIR--NRRL B-15974. Deposited on Jun. 6, 1985.

2. The protein gene expression product of claim 1 consisting of Bacillus**thuringiensis** kurstaki .delta.-endotoxin having the following 610amino acid sequence:
MET ASP ASN ASN PRO ASN ILE ASN GLU CYS ILE PRO TYR ASN CYS LEU SER ASN PRO GLU VAL GLU VAL LEU GLY GLY GLU ARG ILE GLU THR GLY TYR THR PRO ILE ASP ILE SER LEU SER LEU THR GLN PHE LEU LEU SER GLU PHE VAL PRO GLY ALA GLY PHE VAL LEU GLY LEU VAL ASP ILE ILE TRP GLY ILE PHE GLY PRO SER GLN TRP ASP ALA PHE LEU VAL GLN ILE GLU GLN LEU ILE ASN GLN ARG ILE GLU GLU PHE ALA ARG ASN GLN ALA ILE SER ARG LEU GLU GLY LEU SER ASN LEU TYR GLN ILE TYR ALA GLU SER PHE ARG GLU TRP GLU ALA ASP PRO THR ASN PRO ALA LEU ARG GLU GLU MET ARG ILE GLN PHE ASN ASP MET ASN SER ALA LEU THR THR ALA ILE PRO LEU PHE ALA VAL GLN ASN TYR GLN VAL PRO LEU LEU SER VAL TYR VAL GLN ALA ALA ASN LEU HIS LEU SER VAL LEU ARG ASP VAL SER VAL PHE GLY GLN ARG TRP GLY PHE ASP ALA ALA THR ILE ASN SER ARG TYR ASN ASP LEU THR ARG LEU ILE GLY ASN TYR THR ASP HIS ALA VAL ARG TRP TYR ASN THR GLY LEU GLU ARG VAL TRP GLY PRO ASP SER ARG ASP TRP ILE ARG TYR ASN GLN PHE ARG ARG GLU LEU THR LEU THR VAL LEU ASP ILE VAL SER LEU PHE PRO ASN TYR ASP SER ARG THR TYR PRO ILE ARG THR VAL SER GLN LEU THR ARG GLU ILE TYR THR ASN PRO VAL LEU GLU ASN PHE ASP GLY SER PHE ARG GLY SER ALA GLN GLY ILE GLU GLY SER ILE ARG SER PRO HIS LEU MET ASP ILE LEU ASN SER ILE THR ILE TYR THR ASP ALA HIS ARG GLY GLU TYR TYR TRP SER GLY HIS GLN ILE MET ALA SER PRO VAL GLY PHE SER GLY PRO GLU PHE THR PHE PRO LEU TYR GLY THR MET GLY ASN ALA ALA PRO GLN GLN ARG ILE VAL ALA GLN LEU GLY GLN GLY VAL TYR ARG THR LEU SER SER THR LEU TYR ARG ARG PRO PHE ASN ILE GLY ILE ASN ASN GLN GLN LEU SER VAL LEU ASP GLY THR GLU PHE ALA TYR GLY THR SER SER ASN LEU PRO SER ALA VAL TYR ARG LYS SER GLY THR VAL ASP SER LEU ASP GLU ILE PRO PRO GLN ASN ASN ASN VAL PRO PRO ARG GLN GLY PHE SER HIS ARG LEU SER HIS VAL SER MET PHE ARG SER GLY PHE SER ASN SER SER VAL SER ILE ILE ARG ALA PRO MET PHE SER TRP ILE HIS ARG SER ALA GLU PHE ASN ASN ILE ILE PRO SER SER GLN ILE THR GLN ILE PRO LEU THR LYS SER THR ASN LEU GLY SER GLY THR SER VAL VAL LYS GLY PRO GLY PHE THR GLY GLY ASP ILE LEU ARG ARG THR SER PRO GLY GLN ILE SER THR LEU ARG VAL ASN ILE THR ALA PRO LEU SER GLN ARG TYR ARG VAL ARG ILE ARG TYR ALA SER THR THR ASN LEU GLN PHE HIS THR SER ILE ASP GLY ARG PRO ILE ASN GLN GLY ASN PHE SER ALA THR MET SER SER GLY SER ASN LEU GLN SER GLY SER PHE ARG THR VAL GLY PHE THR THR PRO PHE ASN PHE SER ASN GLY SER SER VAL PHE THR LEU SER ALA HIS VAL PHE ASN SER GLY ASN GLU VAL TYR ILE ASP ARG ILE GLU PHE VAL PRO ALA GLU LYS HIS.

US PAT NO: H 875 [IMAGE AVAILABLE] L7: 26 of 27
TITLE: Toxin-encoding nucleic acid fragments derived from a Bacillus **thuringiensis** subsp. israelensis gene

SUMMARY:BSUM(3)
The present invention pertains to novel nucleic acid fragments coding for insecticidal proteins. More specifically, the invention relates to novel fragments encoding insecticidal proteins, said proteins having greater solubility characteristics, less haemolytic activity, and/or greater expression potential in certain specific cells, than the protein encoded by the wild type 27 kDa Bacillus **thuringiensis** var. israelensis gene, the encoded proteins, insecticidal compositions containing these proteins, and th use of these proteins in combating insects, particularly mosquitoes, are also contemplated in the subject invention. Chimeric genes containing the novel nucleic acid fragments, and microorganisms, plant cells, plant tissues, seeds and plants incorporating the nucleic acid fragments are urther within the ambit of the present invention.

BSUM(4)
The spore-forming bacteria Bacillus **thuringiensis** var. israelensis produces a proteinaceous crystalline inclusion which is toxic to the larvae of Mosquito News, 37: 355-358 (1977); de Barjac et al., CR Acad. Sci. Paris, ser D 286: 797-800 (1978); Thomas, et al., FEBS Letters, 154: 362-368 (1983). The native var. israelensis crystal is irregular in shape and consists of several major polypeptides in addition to a number of other polypeptides which are present in minor amounts. See, Thomas et al., J. of Cell Sci., 60: 181-197 1983). A protein of molecular weight 27 kDa is the most prominent of these polypeptides, and its larvicidal and haemolytic properties have been studied using both purified preparations of the 27 kDa .delta.-endotoxin and a 25 kDa segment thereof. See, Davidson et al., Curr. Microbiol., 11: 171-174 (1984); Thomas, W. E. , Ph.D., Thesis, University of Cambridge, "Biochemistry and Mode of Action of the Insecticidal .delta.-endotoxins of Bacillus **thuringiensis**" (1984); Armstrong, et al., J. Bacteriol., 161: 39-46 (1985); Wu et al., FEBS Letts., 190: 232-236 (1985); Lee et al., Biochem. Biophys. Res. Commun., 126: 953-960 (1985); Hurley et al., Biochem. Biophys. Res. Commun., 126: 961-965 (1985); Sriram et al., Biochem. Biophys. Res. Commun., 132: 19-27 (1985); and Cheung et al., Curr. Microbiol., 12: 121-126 (1985).

BSUM(5)
Using a somewhat different approach to investigate the properties of this polypeptide, the gene encoding the 27 kDa .delta.-endotoxin has been cloned in both Escherichia coli (see, Ward et al., FEBS Letts., 175: 377-781 (1984); Waalwijk et al., Nucleic Acids Res., 13: 8201-8217 (1985); Bourgouin et al., Mol. Gen. Genet., 205: 390-397 (1986), and in sporogenic and asporogenic strains of Bacillus subtilis (see, Ward et al., J. Mol. Biol., 191: 1-11 (1986); Ward et al., J. Mol. Biol., 191: 13-22 (1986)). In E. coli, induction of a high level of wild type 27 kDa .delta.-endotoxin expression has been found to have a significant deleterious effect on the growth of that bacterium. It has been postulated that the observed deleterious effect is due to binding of the toxin to phosphatidyl choline and phosphatidyl ethanolamine lipid receptors in E. coli cell plasma membranes. See, Ward, E. S., Ph.D. Thesis, University of Chambridge, "Molecular Genetics of an Insecticidal .delta.-endotoxin from Bacillus **thuringiensis** var. israelensis" (1988); Thomas, et al., FEBS Letters, 154: 362-368 (1983). In B. subtilis recombinants, the 27 kDa protein accumulates in the cytoplasm as phase bright crystalline inclusions, similar in appearance, but smaller than, the var. israelensis crystal. These inclusions have been purified and shown to consist entirely of 27 kDa .delta.-endotoxin. See, Ward et al., J. Mol. Biol., 191: 1-11 (1986); Ward et al., J. Mol Biol., 191: 13-22 (1986). Testing these inclusions in the absence of other crystal polypeptides, has shown this polypeptide to be both larvicidal and haemolytic. See, Ward et al., J. Mol. Biol., 191: 1-11 (1986); Ward et al., J. Mol. Biol., 191: 13-22 (1986). These results are consistent with those reported by Davidson et al., Curr. Microbiol., 11: 171-174 (1984), Thomas, W. E., Ph.D. Thesis, University of Cambridge, "Biochemistry and Mode of Action of the Insecticidal .delta.-endotoxins of Bacillus **thuringiensis**" (1984), and Armstrong et al., J. Bacteriol., 161: 39-46 (1985), but differ from those of several other groups who did not detect mosquitocidal activity in heir preparations of this protein, such as Wu et al., FEBS Letts. 190: 232-236 (1985), Lee et al., Biochem. Biohphys. Res. Commun., 126: 953-960 (1985), Hurley et al., biochem. Biophys. Res. Commun. 126: 961-965 (1985), and Cheung et al., Curr. Microbiol., 12: 121-126 (1985).

BSUM(6)
The nucleotide sequence of the 27 kDa .delta.-endotoxin has been reported in the literature. See, Waalwijk et al., Nucleic Acids Res., 13: 8207-8217 (1985); Ward et al., J. Mol. Biol., 191: 1-11 (1986). the hydropathyl plot of this protein shows it to be highly hydrophobic, and the protein has been shown to interact with specific plasma membrane phospholipids. See, Thomas et al., FEBS Letts., 145: 362-368 (1983). It has also recently been shown by Knowles et al. Biochem. Biophys. Acta., 924: 509-518 (1987) that this protein shares a common cytolytic mechanism with other B. **thuringiensis** .delta.-endotoxine from other serotypes. Commentators in this field have theorized that these .delta.-endotoxins bind to receptors on the membrane, and subsequently interact with the membrane to create a hole or pore. The generation of these pores is thought to lead to colloid-osmotic lysis, where an inflow of ions si accompanied by water influx, which in turn causes cell swelling followed by lysis. See, Knowles et al. Biochem. Biophys. Acta., 924: 509-518 (1987).

BSUM(7)
The present invention is based on a more detailed understanding of the interaction of the var. israelensis 27 kDa .delta.-endotoxin with target membranes. Through in vitro mutagenesis techniques, specific codon alterations have been directed in the cloned .delta.-endotoxin gene. Various of the mutant proteins have been found to possess greater solubility characteristics, less haemolytic activity, and/or greater expression potential in cells containing significant amounts of phosphatidate-type toxin receptors, than the protein encoded by the wild type 27 kDa Bacillus **thuringiensis** var. israelensis gene.

BSUM(10)
In one aspect, the invention pertains to nucleic acid fragments coding for an insecticidal protein having greater solubility characteristics than the protein encoded by the wild type 27 kDa Bacillus **thuringiensis** var. israelensis gene.

BSUM(28)
In a second aspect, the invention pertains to nucleic acid fragments coding for insecticidal proteins having less haemolytic activity than the protein encoded by the wild type 27 kDa Bacillus **thuringiensis** var. israelensis gene.

BSUM(46)
In a third aspect, the invention pertains to nucleic acid fragments coding for insecticidal proteins having greater expression potential in cells containing significant amounts of phosphatidate-type toxin receptors than the protein encoded by the wild type 27 kDa Bacillus **thuringiensis** var. israelensis gene.

)SUM(66)
The invention further relates, in all three aspects, to microorganisms containing these novel nucleic acid fragments, and the use of these novel nucleic acid fragments to modify the properties or characteristics of microorganisms. With respect to the first aspect of he invention, the preferred microorganisms are Bacillus magaterium, Bacillus subtilis and Bacillus **thuringiensis**. The preferred microorganism in the second and third aspect to the invention is Escherichia coli.

)ETDESC:DETD(13)
The phrase "chimeric gene" as employed herein refers to a hybrid construct comprising (1) a nucleic acid fragment in accordance with the present invention which encodes an insecticidal protein and (2) at least one nucleic acid fragment from a different source. Preferably the nucleic acid fragment(s) from a different source comprises a promoter, although it can also include, for example, nucleic acid fragments from other Bacillus **thuringiensis** toxin genes of subspecies israelensis or other subspecies such as aizawai, kurstaki, etc.. Further suitable nucleic acid fragments from different sources will be readily apparent to thoseskilled in the art.

)ETD(19)
The novel insecticide-encoding nucleic acid fragments of the present invention may be obtained from a starting material of wild type Bacillus **thuringiensis** subsp. israelensis using the techniques of genetic engineering, molecular cloning and mutageneis described herein andvariations thereof. Suitable variations on such techniques will be readily apparent to those skilled in the art. For general references on engineering and cloning procedures, see Maniatis et al., "Molecular Cloning: A Laboratory Manual" (Cold Spring Harbon, 1982). A strain of wild type Bacillus **thuringiensis** subsp. israelensis carrying a wild type 27 kDa gene has been deposited with the National Collections of Industrial & Marine Bacteria, Ltd., Torry Research Station, P.O. Box No. 31, 135 Abbey Road, Aberdeen AB9 8DG Scotland, and bears the deposit accession number NCIB 12699. It should also be noted that Bacillus **thuringiensis** subsp. morrisoni PG14 contains a 27 kDa gene which produces a protein quite substantially homologous to the 27 kDa gene product of subsp. israelensis, the encoded protein showing only a single amino acid difference. See, Earp et al., Nucleic Acids Research, 15: 3619 (1987). This would provide a further suitable starting material for the present invention.

)ETD(20)
In one aspect, the invention pertains to nucleic acid fragments coding for insecticidal proteins having greater solubility characteristics than the protein encoded by the wild type 27 kDa Bacillus **thuringiensis** var. israelensis gene.

)ETD(22)
In a second aspect, the invention pertains to nucleic acid fragments coding for insecticidal proteins having less haemolytic activity than the protein encoded by the wild type 27 kDa Bacillus **thuringiensis** var. israelensis gene. The removal or lessening of haemolytic activity has clear advantages, including minimization of any potential mammalian toxicity problems as well as minimization of public concern over the use of this protein in the environment, both of which are often problems and concerns concommitant with the use of agents that show haemolytic tendencies. However, as a practical matter, one skilled in the art would recognize that only under certain select conditions would the haemolytic activity of the subject wild type protein actually translate into a mammalian toxicity problem.

)ETD(23)
In a third aspect, the invention pertains to nucleic acid fragments coding for insecticidal proteins having a greater expression potential in cells containing significant amounts of phosphatidate-type toxin receptors than the protein encoded by the wild type 27 kDa Bacillus **thuringiensis** var. israelensis gene. Preferably, the cells containing significant amounts of phosphatidate-type toxin receptors are E. coli cells. This discovery permits effective production of the insecticidal protein in a number of cells, including E. coli which is one of the most conveniently employed and manipulated organisms presently known to man.

)ETD(55)
The strains of E. coli utilized as cloning hosts for both the wild type 27000 Da .delta.-endotoxin Bacillus **thuringiensis** var. israelensis gene and the mutant derivatives were E. coli TG1 (K12, .alpha.(lac-pro), supE, thi, hsdD5/F'traD36, proA+B+, lacI.sup.q, lacZ.DELTA.M15), available from Dr. T. J. Gibson, MRC Laboratory of Molecular Biology, Cambridge, England and described in Gibon, T. J. Ph.D. Thesis, University of Cambridge, "Studies on the Epstein-Bar Virus Genome" (1984), and E. coli BMH 71-18 Δlac-proAB), thi, supE, F'lacI sup.1, lacZ.DELTA.M15, proA+B+ mutL, available from Dr. G. Winter, MRC Laboratory of Molecular Biology, Cambridge, England and described in Kramer et al., Nucleic Acids Res., 12: 9441-9456 (1984). B. subtilis 168 Sueoka trpC2, available from Dr. T. Leighton, Department of Microbiology and Immunology, University of California, Berkeley, Ca. 94720, and described in Leighton et al., J. Biol. Chem., 246: 3189-3195 (1971), and B. subtilis MB24 metc3, rif, trpC2, available from Dr. P. Piggot, Department of Microbiology and Immunology, Temple University School of Medicine, Philadelphia, Pa. 19140, were also used as cloning hosts for preparation of the wild type 27000 Da .delta.-endotoxin and the mutant derivatives.

)ETD(62)
Site-Directed Mutagenesis of the Bacillus **thuringiensis** subsp. israelensis 27 kDa .delta.-Endotoxin Gene and Expression of the Resultant Mutated Nucleic Acid Fragments

)ETD(63)
The use of an M13 phage vector as a source of single-stranded DNA template has been previously described in Gillam et al., Gene, 8: 81-97 (1979), Gillam et al., Gene, 8: 99-106 (1979), and Winter et al., Nature (London), 299: 756-758 (1982). A 790 bp or 425 bp PstI fragment, containing a portion of the .delta.-endotoxin gene and either 5' or 3' flanking regions were generated using a PstI site in the 27 kDa .delta.-endotoxin Bacillus **thuringiensis** var. israelensis genome and a PstI site in the polylinker of the cloning vector pUC12 (described by Messing, J. Meths. Enzymol., 101: 20-78 (1983). These two fragments were purified and ligated into the PstI site of phages M13tg130 in both orientations, and recombinants producing single stranded DNA (ssDNA) containing the non-coding delta.-endotoxin strand was used as a template

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L1 3540 THURINGIENSIS
L2 11 CRYIE AND CRYIC
L3 11 L1 AND L2

L3 ANSWER 1 OF 11 CAPLUS COPYRIGHT 1997 ACS
TI Bioinsecticides in polymer matrix

L3 ANSWER 2 OF 11 CAPLUS COPYRIGHT 1997 ACS
TI Interactions of Bacillus ***thuringiensis*** crystal proteins with the midgut epithelial cells of Spodoptera frugiperda (Lepidoptera: Noctuidae)

L3 ANSWER 3 OF 11 CAPLUS COPYRIGHT 1997 ACS
TI Spodoptera littoralis (Lepidoptera: Noctuidae) resistance to ***CryIC*** and cross-resistance to other Bacillus ***thuringiensis*** crystal toxins

L3 ANSWER 4 OF 11 CAPLUS COPYRIGHT 1997 ACS
TI Toxicity of Bacillus ***thuringiensis*** spore and crystal protein to resistant diamondback moth (Plutella xylostella)

L3 ANSWER 5 OF 11 CAPLUS COPYRIGHT 1997 ACS
TI Hybrid toxins of Bacillus ***thuringiensis***

L3 ANSWER 6 OF 11 CAPLUS COPYRIGHT 1997 ACS
TI Development of Bacillus ***thuringiensis*** ***CryIC*** resistance by Spodoptera exigua (Huebner) (Lepidoptera: Noctuidae)

L3 ANSWER 7 OF 11 CAPLUS COPYRIGHT 1997 ACS
TI Recombinant Bacillus ***thuringiensis*** crystal proteins with new properties: possibilities for resistance management

L3 ANSWER 8 OF 11 CAPLUS COPYRIGHT 1997 ACS
TI Toxicity of activated CryI proteins from Bacillus ***thuringiensis*** to six forest lepidoptera and Bombyx mori

L3 ANSWER 9 OF 11 CAPLUS COPYRIGHT 1997 ACS
TI Activity of insecticidal crystal proteins and strains of Bacillus ***thuringiensis*** against Spodoptera exempta (Walker)

L3 ANSWER 10 OF 11 CAPLUS COPYRIGHT 1997 ACS
TI Insecticidal properties of a crystal protein gene product isolated from Bacillus ***thuringiensis*** subsp. kenyae

L3 ANSWER 11 OF 11 CAPLUS COPYRIGHT 1997 ACS
TI A novel Bacillus ***thuringiensis*** gene encoding a Spodoptera exigua-specific crystal protein

L3 ANSWER 5 OF 11 CAPLUS COPYRIGHT 1997 ACS
AN 1995:712100 CAPLUS DN 123:249035
TI Hybrid toxins of Bacillus ***thuringiensis***
IN Bosch, Hendrik Jan; Stiekema, Willem Johannes
PA Sandoz Ltd., Switz.; Sandoz-Patent-GmbH; Sandoz-Erffindungen Verwaltungsgesellschaft mbH
SO PCT Int. Appl., 65 pp. CODEN: PIXXD2
PI WO 9506730 A1 950309
DS W: AU, BR, CA, CZ, HU, JP, KR, PL, RU, SK, US RW: AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE AI WO 94-EP2909 940901PRAI GB 93-18207 930902 DT Patent LA English
AB A hybrid toxin of Bacillus ***thuringiensis*** is provided, which hybrid toxin is comprised of a C-terminal domain III of a 1st cry gene (e.g. ***cryIC***) and an N-terminal domain of a 2nd cry protein. Construction of hybrid toxins of cryIA/ ***cryIC*** and ***cryIE*** / ***cryIC*** of B. ***thuringiensis*** was shown. The N-terminal domain may also be selected from other cry proteins such as cryIA(a), cryIA(b), cryIA(c), etc.

L3 ANSWER 7 OF 11 CAPLUS COPYRIGHT 1997 ACS
AN 1994:648597 CAPLUS DN 121:248597
TI Recombinant Bacillus ***thuringiensis*** crystal proteins with new properties: possibilities for resistance management
AU Bosch, Dirk; Schipper, Bert; van der Kleij, Hidle; de Maagd, Ruud A.; Stiekema, Willem J.
CS Dep. Molecualr Biology, DLO-Center Plant Breeding Reproduction Res., Wageningen, 6700 AA, Neth.
SO Bio/Technology (1994), 12(9), 915-18 CODEN: BTCHDA; ISSN: 0733-222X DT Journal LA English
AB To obtain Bacillus ***thuringiensis*** crystal protein with new properties and to identify the regions involved in insecticidal activity, the authors generated hybrid genes composed of ***cryIC*** and ***cryIE*** by in vivo recombination. Anal. of the hybrid proteins showed that domain III of ***CryIC*** is involved in the toxicity towards Spodoptera exigua and Mamestra brassicae. Transfer of this domain to ***CryIE*** , which is not active against these insects, resulted in a new protein with a broader activity. This hybrid protein binds to different receptors than ***CryIC*** , suggesting its use as an alternative for ***CryIC*** in resistance management programs.

L3 ANSWER 11 OF 11 CAPLUS COPYRIGHT 1997 ACS
AN 1991:136871 CAPLUS DN 114:136871
TI A novel Bacillus ***thuringiensis*** gene encoding a Spodoptera exigua-specific crystal protein
AU Visser, Bert; Munsterman, Ellie; Stoker, Andries; Dirkse, Wim G.
CS Cent. Plant Breed. Res., Wageningen, 6700 AA, Neth.
SO J. Bacteriol. (1990), 172(12), 6783-8 CODEN: JOBAAY; ISSN: 0021-9193 DT Journal LA English
AB Only 1 of the 4 lepidoptera-specific crystal protein subclasses (***CryIC***) of B. ***thuringiensis*** was previously shown to be highly toxic against several Spodoptera species. By using a ***cryIC*** -derived nucleotide probe, DNA from 25 different strains of B. ***thuringiensis*** was screened for the presence of homologous sequences. A putative crystal protein gene, considerably different from the ***cryIC*** gene subclass, was identified in the DNA of strain 4F1 (serotype kenyae) and cloned in Escherichia coli. Its nucleotide sequence was detd. and appeared to contain several features typical for a crystal protein gene. Furthermore, the region coding for the N-terminal part of the putative toxic fragment showed extensive homol. to subclass cryIA sequences derived from gene BtII, whereas the region coding for the C-terminal part appeared to be highly homologous to the ***cryIC*** gene BtVI. With an anti-crystal protein antiserum, a polypeptide of the expected size could be demonstrated in Western immunoblots, onto which a lysate of E. coli cells harboring the putative gene, now designated as BtXI, had been transferred. Cells expressing the gene appeared to be equally toxic against larvae of Spodoptera exigua as recombinant cells expressing the BtVI (***cryIC***)-encoded crystal protein. However, no toxicity against larvae of Heliothis virescens, Mamestra brassicae, or Pieris brassicae could be demonstrated. The nucleotide sequence anal. and the toxicity studies showed that this novel crystal protein gene falls into a new cryI gene subclass. It is proposed that this subclass be referred to as ***cryIE*** .